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THE GOITROGENICITY OF MILK FROM COWS CONSUMING CANADIAN RAPESEED MEAL

by

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research for acceptance, a thesis entitled "The Goitrogenicity of Milk from Cows Consuming Canadian Rapeseed Meal," submitted by Donald William Taylor, in partial fulfilment of the requirements for the degree of Master of Science in Animal Biochemistry.

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ABSTRACT

In a series of three feeding trials, milk from cows consuming rapeseed meal, and milk from cows consuming faba beans, were compared for their ability to cause thyroid inhibition in growing rats. By varying the iodine level in the cow and rat feeds, the ability of iodine to overcome any thyroid inhibition caused by the milks was studied.

The results indicated that there were no overall differences between the milk groups in feed or milk consumption or body weight gains. The milk from the cows fed rapeseed meal was lower in iodine and caused significantly larger thyroid weights, abnormal radioiodine uptakes, and shortened thyroid radioiodine half-lives. It also caused insignificantly lower serum PBI and serum T-4 concentrations and elevated serum T-3 and serum T-3/T-4 values.

Many of the goitrogenic effects of the milk from cows consuming rapeseed meal were due to a relative iodine deficiency, but certain indirect evidence indicated the presence of thiocyanate and thionamide goitrogens.

The implications of the above findings on dairy cow and human nutrition are discussed along with some recommendations.

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INTRODUCTION

The recent report of Nutrition Canada (1973) stated that there was an alarming increase in the incidence of enlarged thyroids among Canadians, although urinary iodine excretion indicated that iodine consumption was sufficient. The fact that the majority of these problems occurred in the prairies where most of the rapeseed is grown and much of it marketed, points out that the goitrogenic properties of this crop may be affecting humans. Although milk is not the only foodstuff produced by animals which are fed rapeseed meal, it may have some goitrogenic potential particularly since reports from other countries have already implicated milk from animals fed rapeseed meal (Iwarsson and Nilsson, 1973) and other Brassica sources (Clements and Wishart, 1956; Peltola, 1960) with thyroid problems.

This thesis was undertaken to examine the possibility that milk from cows fed Canadian rapeseed meal may cause inhibition of thyroid function in rats and if so, whether the goitrogenicity is due to an iodine deficiency in the milk or the transfer of a goitrogenic factor to it.

LITERATURE REVIEW

STRUCTURAL, PHYSIOLOGICAL, AND BIOCHEMICAL ASPECTS OF THE THYROID

Structure and Function

The thyroid is an H-shaped gland consisting of two pinkish lobes joined by an isthmus. A connective tissue sheath fixes the gland to the trachea posterior to the larynx, and sends septa into the lobes to lend support and conduct vascular and neural supplies. Microscopically, the cells are arranged into numerous follicles, each follicle being made up of follicular cells surrounding a central lumen which contains a colloid material believed to be the protein-iodine complex, thyroglobulin.

The primary function of the thyroid is to produce the thyroid hormones. Structurally these are among the simplest hormones, consisting of thyronine moieties (two ether-linked tyrosine residues) iodinated in specific positions. Although many iodinated thyronines are possible, the only two showing appreciable physiological activity are 3, 5, 3'-triiodothyronine (T-3) and 3, 5, 3', 5' - tetraiodothyronine (T-4), the former being much more potent on a weight basis (Pitt-Rivers and Rall, 1961).

The thyroid hormones play an important role in maintaining normal calcium, phosphorous, lipid, carbohydrate, and protein metabolism (Wayne, Koutras, and Alexander, 1964) but the actual mechanisms by which these tasks are carried out at the cellular level are obscure. The thyroid hormones do increase cyclic 3', 5' - AMP levels but they do not appear to operate at fixed receptor sites to modify adenyl cyclase activity as many other hormones do (Robison, Butcher and Sutherland, 1971). They may act to regulate the concentrations of intermediates within the cyclic 3', 5' - AMP cascade of control for it is known that a certain background level of thryoid hormones is required for other hormones to display full activity.

Metabolism of Iodine

Iodine is often the limiting factor in the biosynthesis of thyroid hormones, though some recent evidence suggests that iodine is not necessary for the thyroid hormone-like activity of certain synthetic analogues (Goldfine et al., 1974). Why a relatively rare element should be chosen by natural selection for so vital a role is puzzling, but such being the case, a general review of iodine metabolism is in order.

Iodine is present in food and water mainly as iodine (I°) or iodide (I^-) but before absorption can take place, the oxidized forms must be reduced to iodide (Cohn, 1932). There appears to be ample intestinal ability to do this since absorption problems are rarely linked to iodine deficiency. Once absorbed, the iodide enters the plasma inorganic iodide (PII) pool which is normally below 0.1 µg/100 ml (Tong, 1971).

Since most tissues other than erythrocytes (Myant et al., 1950) are impermeable to iodide, little iodide is removed except by the organs actively concentrating it. Besides the thyroid and kidney, the mammary, salivary, and gastric glands are able to maintain a positive iodide concentration gradient utilizing a basically similar trapping mechanism. The thyroid however is the most efficient in this respect with thyroid: serum iodide concentration ratios in excess of 100 being recorded under certain circumstances (Halmi, 1954).

Biosynthesis and Secretion of Thyroid Hormones

Once iodide has entered the thyroid gland, biosynthesis of the thyroid hormones is essentially a three stage procedure. Initially the iodide is oxidized to iodine using hydrogen peroxide and the enzyme, iodide peroxidase (Tong, 1971). This is followed by the iodination of the tyrosine residues of thyroglobulin to form 3- or 5- monoiodotyrosine (MIT) and 3, 5- diiodotyrosine (DIT). Although an enzyme is not required in vitro (Taurog, 1955), the hypothetical tyrosine iodinase is believed to mediate this step in vivo to direct iodination towards thyroglobulin and not other proteins. Finally, the coupling of two iodinated tyrosines takes place to form T-3 or T-4. This step is carried out within the thyroglobulin structure and likely involves a peroxidase enzyme system (Taurog, 1968).

The newly iodinated thyroglobulin is extruded into the follicular lumen for storage. The total thyroid storage of synthesized hormone is quite extensive since an animal may be able to maintain normal thyroid hormone output for several weeks after the synthesis of new hormone has been blocked (Tepperman, 1973). Secretion is accomplished by resorbing the most recently iodinated thyroglobulin (Schneider, 1964) back into the follicular cell where proteolysis takes place (Laver and Trikojus, 1956). The free iodothyronines are discharged into the extracellular fluid while any free iodotyrosines are rapidly deiodinated by iodotyrosine deiodinase (Taurog, Porter, and Thio, 1964), and the iodide is quickly reutilized to iodinate more tyrosine residues of thyroglobulin.

Plasma Thyroid Hormone Transport and Degradation

Most of the circulating plasma thyroid hormone is found attached by non-covalent bonds to plasma proteins. T-4 is bound quite strongly by thyroxine-binding globulin (TBG), thyroxine-binding prealbumin (TBPA), and albumin, while T-3 is bound less firmly by TBG and albumin (Oppenheimer and Surks, 1971). As it is only the free hormone that is metabolically active (Robbins and Rall, 1957) and since probably less than 1% of the hormone is in the unbound state, fluctuations in the thyroxine-binding protein (TBP) levels could affect an animal's thyroid hormone activity status more than changes in the hormone concentrations. Under normal conditions, TBP concentrations are quite constant but certain compounds including the estrogens will raise TBP concentrations while the androgens will depress them (Engbring and Engstrom, 1959). Under abnormal TBP conditions, the absolute level of thyroid hormone may be altered to ensure normal free hormone concentrations.

The thyroid hormones undergo peripheral degradation in a number of ways, the most important being deiodination which occurs in the liver, kidney, muscle, brain, and heart (Oppenheimer and Surks, 1971). The iodothyronines are eventually deiodinated to varying degrees yielding iodide for the PII pool and thyronines in different stages of iodination. The fate of these thyronine compounds is quite complex, but many are conjugated (mainly with glucuronic acid) and excreted in the bile (Taurog, Briggs, and Chaikoff, 1952).

One important product of the partial deiodination of T-4 is T-3. Although the plasma concentration of T-3 is only 3% of T-4, T-3 is turned over much more rapidly giving a daily clearance rate almost as great as T-4 (Woeber et al., 1970). Since T-3 is three- to four- times as active as T-4 on a weight basis (Robbins and Rall, 1967), as much as two-thirds of the total thyroid hormone effect may be due to T-3.

Estimates of T-4 to T-3 conversion reveal that 30-35% of the total T-3 may arise from the extrathyroidal degradation of T-4 (Sterling, 1970; Pittman, Chambers, and Read, 1971; Sterling, Brenner, and Neuman, 1970). Some researchers have speculated that T-4 must be converted to T-3 to have thyroid hormone activity. This concept is consistent with the finding that liver problems leading to a failure in the extrathyroidal conversion of T-4 to T-3, may also lead to hypothyroidism (Nomura and Pittman, 1974). Goldfine et al. (1974) however, have found that not all of the T-4 activity can be explained by its conversion to T-3.

Regulation of Thyroid Hormone Secretion

Thyroid hormone secretion is increased by thyroid-stimulating hormone (TSH) which in turn is controlled by thyrotropin releasing factor (TRF) from the hypothalamus (Reichlin, 1971). The free T-3 and T-4 levels in the plasma have a negative feedback effect on both TSH secretion (Degroot, 1971) and TRF secretion (Tepperman, 1973). TSH stimulates thyroid hormone secretion by affecting almost every step involved in thyroid iodine metabolism including iodide trapping (Halmi et al., 1960), organic iodinations (Tong, 1964), iodotyrosine coupling (Shimoda, Kendall, and Greer, 1966), and hormone secretion (Rosenberg, Athans, and Behar, 1960).

TSH is also capable of causing thyroid hypertrophy (Nadler, 1971) and possibly hyperplasia (Tepperman, 1973), resulting in increased thyroid size or goitre. This is an important adaptation since unlike chloride regulation, there is no adaptable renal iodide clearance rate to maintain a constant plasma level (Wayne et al., 1964). Since the PII fluctuates with dietary iodine intake, it is the thyroid clearance of plasma iodide which must be regulated. This is achieved by a combination of increased iodide trapping efficiency and greater thyroid size to enable a larger volume of plasma to be cleared of its iodide content per unit time (Wayne et al., 1964).

PATHOLOGICAL ASPECTS OF THYROID FUNCTION

There are several conditions that interfere with normal thyroid function. This review will only consider goitrogenic compounds and iodine deficiency since they are pertinent to this study.

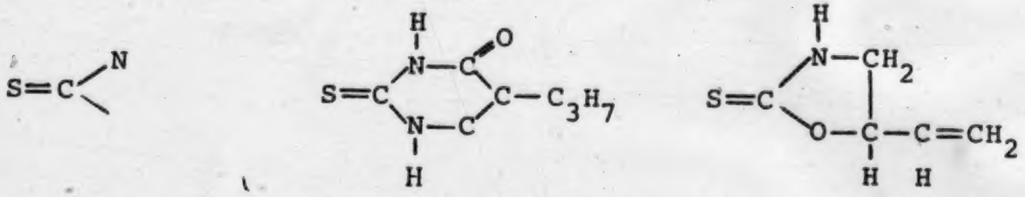
Mechanism of Action of Goitrogens

Goitrogens, or compounds which produce inhibition of thyroid function, have been classified according to their structure and mode of action (Greer, Kendall, and Smith, 1964; Green, 1971) but only those groups containing compounds known to be found in rapeseed meal will be discussed here.

The first class of antithyroid agents are the monovalent anions, the principal members being perchlorate and thiocyanate. All of these compounds are similar in electrical charge and molecular size to iodide (Wolff, 1964) and so compete for active uptake. Since competitive inhibition is readily overcome by a simple mass action effect, increasing the dietary iodine intake will remove the thyroid inhibition caused by these compounds (Astwood, 1943; Greer, 1950).

Thiocyanate may also interfere with thyroidal iodinations by serving as a substrate for iodide peroxidase (Maloof and Soodak, 1966). This effect may not be physiologically meaningful however, since it occurs at thiocyanate concentrations above those necessary for iodide transport inhibition (Greer, Stott, and Milne, 1966).

The second class of antithyroid agents are the thionamides (Figure 1). These compounds affect several of the reactions of thyroid hormone synthesis with the order of susceptibility being coupling of iodothyronines (most susceptible), iodination of MIT, and iodination of tyrosine (Richards and Ingbar, 1959; Lo and Hill, 1971).



Thionamide Nucleus

Propylthiouracil

Goitrin (found in rapeseed)

FIGURE 1. Thionamide Goitrogens

The exact mechanisms of action of the thionamide goitrogens are not well understood and the evidence as to the effectiveness of iodine prophylaxis is conflicting. When large amounts of thionamide goitrogens are consumed, hormone synthesis is completely blocked and no amount of supplementary iodine can remedy the situation (Greer, 1962). With small dosages however, Greer et al. (1962) suggest that any goitrogenic effects of propylthiouracil could be negated with iodine additions to the diet. This suggestion is in conflict with the findings of Finnish researchers who noted that enlarged thyroids persisted even when excess iodine was administered to rats consuming small amounts of mercazole (Krusius and Peltola, 1963) or goitrin (Krusius and Peltola, 1966).

Iodine Deficiency

Goitrogenic compounds are important in the etiology of thyroid problems, but it is estimated that 96% of the cases of human non-toxic goitre are due to simple iodine deficiency (Van Etten, 1969). Much of this goitre has traditionally occurred in the areas which had most of their iodine removed by the meltwaters of the last glacial recession (Kelly and Snedden, 1960).

Thyroidal inhibition due to iodine deficiency reveals definite sex differences with females being much more susceptible (Nutrition Canada, 1973; Koutras, 1971). The iodine losses during pregnancy, lactation, and menstruation account for much of this, but there is also a more direct hormonal aspect. Grosvenor (1962) found that estrogens caused increased fecal losses of thyroid hormone in rats and this combined with the increased TBP levels may indicate that less free hormone is available for a given iodine intake in females.

Animals have developed several mechanisms to maintain normal serum thyroid hormone levels under an iodine-deficient dietary regime. Most of these are mediated by TSH and have been discussed, but of the remaining methods, organic iodine storage is the most often overlooked. Less than half of the iodotyrosines produced become iodothyronines, meaning that for every iodothyronine released there are two iodotyrosines deiodinated for reuse. Although inefficient, this process does mean that during periods of excess iodine, large amounts of it are kept bound in an organic molecule and out of the equilibrium with PII.

Another important method of iodine deficiency adaptation involves a shift in the thyroid hormone production pattern to favour T-3 (Greer, Grimm, and Struder, 1968) resulting in only 75% of the iodine being required to produce a more potent compound. Although the cellular mechanisms aren't understood, continuous TSH secretion may be the regulating factor (Forest et al., 1974).

DIAGNOSIS OF THYROID INHIBITION

The diagnosis of thyroid problems requires a wide range of parameters to enable accurate conclusions to be drawn on the severity of the stress present. For the purpose of this thesis, it is proposed that these parameters be grouped according to whether they are used to diagnose mild (compensated) goitre or severe (uncompensated) goitre.

Compensated Goitre Parameters

Compensated goitre implies that a thyroid stress is present but that the animal is able to compensate to the extent that it maintains normal plasma thyroid hormone levels (euthyroidism).

Thyroid radioiodine uptake. A sensitive parameter for detecting compensated goitre is thyroid radioiodine uptake. This is carried out by injecting a known amount of radioiodine (^{125}I or ^{131}I) and then at a given time interval, measuring the radioactivity in the thyroid. An animal on an iodine-deficient ration has an increased radioiodine uptake due to its greater efficiency of thyroidal plasma clearance. A thiocyanate type of goitrogen causes low radioiodine uptake because of the inhibition in iodide trapping. The response of an animal on a thionamide goitrogen is variable depending upon the time interval after radioiodine injection. Since iodide trapping isn't blocked, uptake during the first 10 minutes is elevated due to the indirect thionamide effect on TSH secretion via circulating T-3 and T-4 concentrations (Thomas, Oddie, and Myhill, 1960). With longer intervals, uptake is usually depressed since the iodine remains inorganic and so is in equilibrium with the PII (Greer et al., 1962). When small amounts of a thionamide goitrogen are ingested, Slingerland et al. (1959) found an occasional but inconsistent elevation in 24-hour radioiodine uptake while Krusius and Peltola (1966) felt that radioiodine uptake was not sensitive enough to detect minute amounts of a thionamide goitrogen in feedstuffs.

Thyroid weights. The thyroid will increase in size by an amount proportional to the stress imposed upon it except in some cases of minor thyroid inhibition when compensation is possible without hypertrophy (Bishopric, Garrett, and Nicholson, 1955). Differential diagnosis between goitrogens and iodine deficiency is difficult however, since both cause an increase in thyroid weight.

Thyroid radioiodine half-life. A third method of monitoring mild thyroid stress is to measure the half-life of a single dosage of radioiodine after it has been taken up by the thyroid. In the rat, this can be done by injecting ip. a known dosage of ^{131}I, waiting for uptake to maximize, and then externally counting the thyroid at different times to determine the radioiodine disappearance rate

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(Reineke and Lorscheider, 1967).

An animal consuming adequate amounts of iodine will have a fairly low radioiodine uptake. Any ^{131}I taken up however, will be synthesized into thyroid hormone and stored in the thyroglobulin resulting in a slow disappearance (long half-life) of the initially measured radioiodine.

An iodine-deficient animal will trap a large percentage of the radioiodine, but since it will be continually resorbing stored hormone, the "last come, first served" principle of Schneider (1964) will dictate that the newly synthesized radioactive hormone be secreted quickly. Once in the plasma, the radioiodine is more subject to renal clearance than thyroidal radioiodine and so despite recycling, the thyroid radioiodine half-life is short.

The effect of goitrogens on thyroidal radioiodine half-life is complicated, but it is assumed that there will be a low radioiodine uptake as well as a short half-life. A thionamide goitrogen interferes with the formation of organic iodine in the thyroid and since inorganic iodine is in equilibrium with PII, there will be a high thyroid radioiodine loss. A thiocyanate goitrogen will not interfere with the organification of ^{131}I, but since TSH is also indirectly stimulated, the radioactive hormone produced will be secreted rapidly.

Other methods. Other methods of measuring minor thyroid inhibition are available but weren't used in this study. Plasma TSH measurement has only recently become clinically feasible (Webster, Paice, and Gale, 1970) and it should be able to detect even the smallest changes in plasma hormone levels, if indeed plasma TSH remains elevated in the compensated state (Wayne et al., 1964). Knowing the PII, the absolute iodide uptake, and the rate of thyroid clearance of plasma iodide (Koutras et al., 1961; Alexander et al., 1962) would be useful, but the sampling of blood and urine from the large number of animals used in this study made these measurements unfeasible.

Uncompensated Goitre Parameters

When the iodine deficiency or goitrogen effect becomes severe, the animal is no longer able to compensate and so becomes hypothyroid.

Serum protein-bound iodine concentrations. Determination of serum protein-bound iodine (PBI) is the simplest method to measure plasma thyroid hormone concentrations. This method is usually carried out by pretreating the plasma with an anion exchange resin to remove the inorganic iodide (Blanquet, Dunn, and Tobias, 1958). After digestion, measurement of total iodide present in the protein fraction is carried out utilizing the iodide catalysed reduction of the ceric ion by arsenite (Sandell and Koltoff, 1937). Although some elements such as mercury interfere with PBI determinations, the procedure is accurate if proper care is taken.

There are two main drawbacks to the PBI method. Firstly, it doesn't measure the actual hormones, but rather all sources of organic iodine including such exogenous contaminants as those in cough remedies, antiseptics, and antiasthmatics (Davis, 1966). Secondly, PBI doesn't distinguish between T-3 and T-4, so an accurate estimate of hormone physiological activity is not derived.

Serum T-3 and T-4 concentrations. To overcome the PBI problems the actual serum concentrations of T-3 and T-4 can be measured. These methods are basically competitive protein-binding assays (Elkins, 1960; Murphy and Pattee, 1964) or their radioimmunoassay modifications (Braverman et al., 1971; Surks, Schadlow, and Oppenheimer, 1972; Larsen, 1972; Lieblick and Utiger, 1972). In principle, a competition for a finite number of binding sites is set up between the plasma hormone and an exogenous source of radioactive hormone. After removing the unbound fraction, the amount of radioactivity present is inversely proportional to the amount of hormone in the plasma sample. These techniques are extremely accurate, the main criticism being that the total serum hormone is measured rather than the free fraction (Selenkow, 1971).

Animal performance characteristics. The final parameters worthy of measurement are certain characteristics of animal performance including growth rate, feed consumption, and reproductive efficiency. In this study it is assumed that only after all of the compensatory mechanisms have failed and the thyroid hormone levels have dropped below those necessary for normal metabolic functioning, will these characteristics be affected.

POTENTIAL GOITROGENICITY OF MILK

Now that thyroid function and some aspects of its pathology have been reviewed, the potential of milk to cause thyroid inhibition must be examined. Although the importance of milk as a source of iodine has declined since the advent of iodized salt and iodized white bread, the possibility that young children may still obtain most of their iodine requirements from milk means that iodine deficiency in milk is as worthy of investigation as the potential goitrogens in milk.

Iodine Requirements of Lactating Cows

In the latest revision of the NRC dairy cattle requirements NRC (1971), the supplementary iodine requirement is set at 0.6 mg/kg DM consumed. This requirement can be met by feeding iodized stock salt (0.015% I as KI) at 1% of the concentrate mix if it is assumed that grain constitutes 40% of DM intake. If a goitrogen is present in the feed, NRC (1971) makes a general but unquantitative recommendation that the iodine level be increased. The ARC (British Agricultural Research Council, 1965) suggests that iodine be included at

0.8 mg/kg DM but that this should be increased to 2.0 mg/kg DM if goitrogenic factors are present.

Iodine Content of Milk

The mammary gland is able to take up iodide and maintain a concentration 30-times greater than the plasma (Honour, Myant, and Rowlands, 1952). Although the mechanism is similar to that of the thyroid, the latter is able to trap much more iodide relative to its size, perhaps because iodide is quickly incorporated into the thyroglobulin molecule (Miller, Swanson, and Cragle, 1965). Milk iodine is in the iodide form or an association of iodine with the milk proteins (Miller and Swanson, 1963; Archibald, 1958).

There are several factors which determine the iodine content of milk but the dietary level and form are the most important. Hemken et al. (1971) found that milk iodine concentrations went from 0.8 µg/100 ml when cows were fed an iodine-deficient diet, up to 69.4 µg/100 ml when cows were receiving the same diet with 68 mg KI per day. Miller and Swanson (1973) found milk iodide levels between 0.8 µg/100 ml and 239.3 µg/100 ml when cows were fed no supplementary iodide and 1000 mg of iodide per day respectively. These authors also found that 100 mg of iodide fed in an organic form such as ethylene diamine dihydroiodide resulted in milk iodine levels twice as high as when the same amount of iodide was fed as KI.

Since higher producing cows secrete a greater amount of iodine in their milk (Miller and Swanson, 1963), the possibility exists that a lactation may become self-limiting due to reduced thyroid function because of the thyroid: mammary competition for iodine (Lorscheider, Oxender, and Reineke, 1969). Swanson (1972) however found that cows on a low iodine diet were able to maintain their thyroid secretion rates by recycling the available iodine more efficiently and secreting less in their milk. These iodine utilization priorities were used by Alderman and Stranks (1967) as the basis for predicting a cow's iodine status from her milk iodine concentration. After correlating the iodine levels of the milk and blood, they estimated that a milk iodine level below 2.5 μg/100 ml signifies iodine deficiency.

Goitrogens of the complex anion group affect milk iodine levels by inhibiting mammary gland uptake (Lengemann, 1965; Garner, Sansom, and Jones, 1960). Piironen and Virtanen (1963) found a decreased milk iodine level when thiocyanates were fed and so recommended higher dietary iodine levels if Brassica plants are consumed. Hemken et al. (1972) made a similar recommendation for soybean meal which also decreases milk iodine levels.

Other factors also influence milk iodine concentrations. Iodine levels increase as production goes down

(Garner et al., 1960) and therefore increase in later lactation (Iwarsson, 1973). Seasonal effects are unlikely (Miller and Swanson, 1963; Garner et al., 1960) although Lengemann, Swanson, and Monroe (1957) have suggested a possible stimulus for iodine secretion in the spring. Finally, the use of an iodophor teat dip was found to increase the milk iodine concentrations by an average of 17.4 µg/100 ml when compared to other dips (Iwarsson and Ekman, 1974).

Goitrogens in Milk

The first suggestion of goitrogenic compounds in milk was that of Clements and Wishart (1956) who found that administering iodine to Tasmanian school children did not decrease the incidence of goitre. Upon further investigation they found that milk from cows which were pastured on marrow-stemmed kale (Brassica olenacea) in this area, produced a depression in radioiodine uptake in humans and rats and so it was assumed that goitrin was getting into the milk. Wright (1958) produced evidence that at least part of the goitrogenic effect of milk from goats which were fed kale was due to thiocyanates although Allcroft and Salt (1961) were unable to find increases in blood or milk thiocyanate concentrations when cows were fed kale.

In Finland, Virtanen, Kreula, and Kiesvaara (1958) fed a cow with 0.5 kg of finely ground, moistened rapeseed (containing 4.5 g of goitrin) and found milk levels of a compound similar to goitrin to be up to 10 μg/100 ml with a total milk secretion of about 0.1% of the goitrin administered. In later trials, this same group (Virtanen, Kreula, and Kiesvaara, 1963) found goitrin levels ranging from 0.4 μg/100 ml to 7.7 μg/100 ml when 30 kg of marrow-stemmed kale (containing 66 mg goitrin) and 100 g of crushed, moistened rapeseeds (containing 800 mg goitrin) respectively were fed. The results showed that only 0.05% of the goitrin fed appeared in the milk and since a single dose of 20 mg is required to cause thyroid inhibition in man, they concluded that goitrin in milk need not be considered as a potential problem.

This same group (Virtanen et al., 1963) also investigated the possibility of thyroid inhibition caused by thiocyanates in milk. They found that cows fed large amounts of Brassica fodder produced milk with a thiocyanate concentration of 0.6-0.9 mg/100 ml. They concluded that these levels would have no effect upon man since a single dosage of 300 mg of thiocyanate is required to depress radioiodine uptake.

Peltola's group, also in Finland, disagree quite strongly with the conclusions drawn by Virtanen et al., (1963). In their experiments (Peltola, 1960), milk from the endemic goitre district of Finland caused significant increases in rat thyroid weights, even when the rats were receiving iodine far in excess of their daily requirements. Similar results were obtained when goitrin was fed to rats at levels corresponding to 1-5 μg/100 ml (Krusius and Peltola, 1966). Since milk from the goitre district was later found to contain goitrin at 3.5 - 10.0 μg/100 ml (Arstila, Krusius, and Peltola, 1969), it was concluded that the thyroid enlargements found in the initial rat trials were due to the goitrin content of the milk.

For interpretation, Krusuis and Peltola (1963) cite the work of Greer who found that goitrin was excreted rather slowly. Thus goitrin in a small but continuous dosage may accumulate in the body to cause serious results. Peltola has estimated, both from studies with mercazole (Krusius and Peltola, 1963) and goitrin (Krusius and Peltola, 1966), that for thionamide goitrogens there is a 1000-fold difference in the smallest single dose necessary to depress thyroid radioiodine uptake, and the smallest chronic dosage required to cause thyroid enlargements. Thus the conclusions of Virtanen et al. (1963) appear to be invalid since they are based upon single dosage results and not daily dosages such as would occur when an animal consumes milk containing a low level of a goitrogen.

The Goitrogenic Properties of Milk from Cows Consuming Rapeseed Meal

Rapeseed is known to contain certain glucosinolates which when hydrolysed in the presence of myrosinase (also present in rapeseed) form goitrin and thiocyanates. Although

80% of the rapeseed grown in Canada is of the low glucosinolate species (Brassica campestris)(Clandinin, Slinger, and Bell, 1972), goitrogens still appear in rapeseed meal (RSM) in sufficient quantities to cause observable symptoms in poultry and swine. These include reproductive problems, increased iodine requirements, slower growth rates, enlarged thyroids, and depressed serum PBI's (Clandinin, Robblee, and Slinger, 1972; Bowland and Bell, 1972).

Unlike monogastrics, ruminants are not too adversely affected by RSM feeding other than by a slight palatability problem (Ingalls and Waldern, 1972). Trials in Canada (Ingalls and Seale, 1971; Ingalls, Seale, and McKirdy, 1968) and in Sweden (Iwarsson, 1973) indicate that up to 10% of the concentrate ration can be RSM without causing depressions in production, reproductive efficiency, or thyroid function.

The possibility that milk from cows fed RSM (RSM-milk) may have some goitrogenic properties was investigated by Iwarsson and Nilsson (1973) in Sweden. In rat feeding trials, these researchers compared milk from cows consuming RSM at 35% and 20% of their oil cake mixtures (8.05% and 4.20% of the concentrate rations respectively) with milk from cows consuming soybean meal (SBM-milk). The results indicated that the rats on the RSM-milk rations had significantly larger thyroids, higher ^{131}I - 24 hour uptakes, and lower PBI's than the rats consuming the SBM-milk ration.

From experiments supplementing the RSM-milk with iodine and the SBM-milk with thiocyanates, they concluded that the main goitrogenic factor was the lowered iodine content of the RSM-milks. The possibility that the milk from the RSM groups contained goitrin was not ruled out since the milk from the cows consuming the higher RSM ration caused lower PBI values than the other RSM-milk, even though the rats consumed the same amounts of iodine. The pattern of radioiodine uptake however, is not similar to that of animals consuming either thiocyanates or goitrin.

Since Canada is the world's largest producer of rapeseed, this study was therefore undertaken to see if the goitrogenic properties found by Iwarsson and Nilsson (1973) in the milk from cows fed Swedish rapeseed meal, exists in the milk from cows fed Canadian rapeseed meal.

OBJECTIVES

The objectives of this study were as follows:

1. To determine whether milk from cows consuming Canadian rapeseed meal has any goitrogenic effects when fed to rats.
2. To assess the ability of iodine supplementation of the cow and rat diets to alleviate possible goitrogenic effects of milk produced on Canadian rapeseed meal.
3. To discover the causes of any rat thyroid inhibition occurring as a result of the consumption of milk produced on Canadian rapeseed meal.

MATERIALS AND METHODS

General

Three separate trials were run in which the milk from RSM-fed cows (RSM-milk) was fed to laboratory rats. The rats were then critically examined using various parameters of thyroid inhibition and the results compared to those of rats consuming control diets.

The liquid control diets consisted of both water and milk controls. The water was used to compare the results of the milk groups to the normal values for laboratory rats. The milk control (FABA-milk) was obtained by feeding faba beans (*Vicia faba* L. vars. *minor*) in place of RSM in the cow diets. Faba beans were chosen partially because they were already being fed to the cows in the University of Alberta herd for other trials, and also because they have not been shown to contain goitrogens (Van Etten, 1969).

The different liquid diets were tested against both sexes (Trials I and II), two experimental time periods (Trial I), and high and low iodine feeds (Trials II and III).

Animals and Diets

Cows. The cows were either Holstein or Holstein X Brown Swiss from the University of Alberta dairy herd housed at the University of Alberta Dairy Research Unit. Each treatment group consisted of three cows to enable pooled milk sampling. The background data for the cows in Trials II and III can be found in Appendices C-1 and C-3 respectively.

The cows producing the experimental milks were treated in a manner identical to that of their herdmates except for the protein supplement and iodine content of their diets. Concentrate consisted of the regular Dairy Research Unit oats-barley mix balanced with the test protein supplements (Table 1). Concentrate and chopped hay were fed in a 40/60 (w/w) ratio to meet NRC (1971) requirements for maintainence and production. The cows were pastured whenever farm management practices would allow. Milking was done twice per day and a non-iodophor teat dip (Hibitane, Ayerst Laboratories, St. Laurent, Quebec) was used to avoid iodine contamination of the milk. Milk samples for rat feeding were taken twice per week, preserved with formaldehyde in a final concentration of 2×10^{-2} M (Murthy and Campbell, 1966), and stored at 4C between feedings. Milk iodine analysis was carried out for each milk sample taken.

The dietary treatment groups by trial were as follows:

(a) Trial I (April 19- May 24). RSM and FABA were the two treatment groups and it was planned that NRC (1971) iodine requirements be met by the inclusion of trace mineralized salt (0.01% I as ethylene diamine dihydroiodide (EDDI)) in the grain ration and free choice mineral mix. Due to an oversight however, the cows were given a high iodine salt (0.15% I as EDDI) for the prevention of foot-rot. Thus the cows were actually getting 15-times NRC (1971) requirements

TABLE 1

FORMULATION OF COW CONCENTRATE RATIONS

Ingredient	RSM (kg)	FABA (kg)	BRON (kg)
Rolled Oats	330	280	330
Rolled Barley	440	390	440
Molasses	30	30	30
Sodium Tri-polyphosphate	6.66	6.66	6.66
Limestone	20	20	20
Vit. A,D,E	0.27	0.27	0.27
Vit. D_3	0.27	0.27	0.27
Shorts	1.13	1.13	1.13
Trace Mineral Salt*	5	5	5
Rapeseed Meal	166.67	-	-
Faba Beans	-	266.67	-
Bronowski Meal	-	-	166.67
TOTAL	1000	1000	1000

*Trace mineralized salt contains 0.01% I
No salt added to grain mix in Trial III

during this trial.

Trial II (August 20 - September 26). The dietary treatments were similar to those in Trial I except a third group (BRON) was formed using the meal produced from the Bronowski low glucosinolate rapeseed variety (Table 1). The iodine requirements were met by the inclusion of trace mineralized salt in the concentrate ration and free-choice mineral mix.

Trial III (November 7 - December 12). As in Trial I, only two types of protein supplement (RSM and FABA) were fed, but no trace mineralized salt was included in either the concentrate ration or free-choice mineral mix. Instead the salt requirements were met by top-dressing with about 50 g twice per day of one of three salt mixes (H, M, or L). The H salt mix was iodized stock salt (0.015%I as KI), the M salt mix was half iodized salt, half non-iodized salt (0.0075% I as KI), and the L salt mix was noniodized salt. Therefore the RSM-H and FABA-H received NRC (1971) supplementary iodine requirements, the RSM-M and FABA-M received half of the NRC (1971) supplementary iodine requirements, and the RSM-L and FABA-L received no supplementary iodine.

Rats

The rats in Trials I and II were of the Sprague-Dawley strain, housed in the Department of Animal Science at the University of Alberta. The rats in Trial III were of the

Charles River CD strain (Canadian Breeding Farms and Laboratories Ltd., St. Constant, Que.) housed in the facilities of Bioscience Animal Services at the University of Alberta. All animal rooms were kept at 22± 1C and the lights were on for 12 hours daily. Cage groups contained four rats, placed on trial as soon after weaning as possible.

Solid feed was offered free-choice in round metal feeders covered with a perforated plate to prevent wastage. The test liquid was fed ad libitum and changed once per day, the bottles being washed between feedings. Cage milk and feed consumption and individual rat weights were recorded on a weekly basis.

Trial I. This trial tests RSM- and FABA- milk across both sexes for a 5 week trial. In addition, the two types of milk were compared using females in a 2 week trial. All rats were fed a low-iodine (0.04 mgI/kg) wheat starch ration (Table 2).

At the end of the trial, the rats were injected ip. with about 2μCi of ^{125}I (Amersham/Searle, Arlington Heights, Ill.) in 1 ml of physiological saline. Exactly 24 hours later they were sacrificed by a blow to the head and bled by heart puncture. The blood was immediately transferred to a heparinized tube, centrifuged, and the plasma frozen and stored at -4C for subsequent PBI analysis. The thyroids were removed and quickly weighed in a sealed plastic container.

TABLE 2

TRIALS I AND II - FORMULATION OF RAT LOW IODINE RATION

Ingredients	g/kg
Ground Wheat	700.00
Corn Starch	298.6
Ferric Ammonium Sulphate	0.07
Cupic Sulphate	0.03
Sodium Chloride	0.9
Vit. A,D,E Premix	0.400

The radioactivity of the thyroids was later measured (Beckman Biogamma, Beckman Instruments Ltd., Fullerton, Calif.) with care being taken to place the thyroids on the bottom of the counting vial to avoid differences in geometry which could affect counting efficiency.

Trial II. This trial tested five types of liquids across two solid feeds and both sexes. The five liquids were RSM-, FABA-, and BRON- milks, WATER, and STORE-milk (pasteurized, 2% B.F. milk obtained from a local grocery store). The two types of solid feed were Low I (Table 2) containing 0.04 mgI/kg and High I (Teklad Rat Diet, Teklad Mills, Madison, Wisc.) containing 2.0 mgI/kg. Due to a shortage of male rats, this trial consisted of only 19 cages, the missing block being the STORE-High I- Male group.

Trial II was terminated much like Trial I except an ip. injection of 25 mg of sodium pentobarbitol (Abbott Laboratories, Montreal, Que.) was used to sacrifice the animals, and the plasma was analysed for T-3 and T-4 rather than PBI.

Trial III. This trial tested water and the six milks (RSM-H, FABA-H, RSM-M, FABA-M, RSM-L, FABA-L) across two modified Remington iodine-deficient diets (Remington, 1937) (Table 3). The Low I feed contained 0.15 mgI/kg and the High I feed contained 0.28 mgI/kg. There were three cages for each of the 14 liquid-feed combinations for a total of 42

TABLE 3

TRIAL III - FORMULATION OF RAT RATION

Ingredients	Amount (Kg/100 kg)	(A) Mineral Mix for 4 Kg	Amount (g)	(B) Vitamin Mix for 4 Kg	Amount (mg)
Shelled, Ground Yellow Corn	78	Calcium Carbonate	1200	Ascorbic Acid	99,000
Wheat Gluten	18	Potassium Di-phosphate	1326	Choline Chloride	165,000
				D-Ca Pantothenate	6,600
Mineral-Vitamin Mix	4	Cupric Sulphate	1.0	Niacin	9,900
				PABA	11,000
		Ferrous Sulphate	72.91	Pyridoxine HCl	2,200
		Magnesium Sulphate	203.27	Riboflavin	2,200
				Thiamine HCl	2,200
		Monocalcium Phosphate	223.01	Vit. A Acetate	1,980,000 IU
		Manganese Sulphate	15.16	Vit. D_3	120,000 IU
				Vit. E	11,000
		Sodium Chloride	630.00	Vit. K	4,950
		Zinc Chloride	1.00	Vit. B_{12}	2.97
				Biotin	44
				Folic Acid	198

cages. The milk feeding portion of the trial was 5 weeks long, preceded by a 7 day milk-free period to accustom the rats to their solid feed diets.

Termination of this trial was somewhat different than Trials I and II. Rat thyroid radioactivity was measured on four separate occasions to determine thyroid radioiodine half-life as outlined below. Following the final measurement, blood was taken by heart puncture from the anesthetized rats, allowed to clot for 20 minutes, centrifuged, and the resulting serum frozen and stored at -4C for later T-3 and T-4 analysis. The thyroids were removed and weighed as in Trial I.

ANALYTICAL TECHNIQUES

Serum Protein-Bound Iodine Concentration

The Hycel Cuvette PBI (Hycel Inc., Houston, Texas) technique was used.

Total Iodine in Milk

This method was similar to the PBI method except no resin pretreatment was carried out.

Total Iodine in Feed

Feed was analysed for iodine by Dr. Karin Thente, National Swedish Laboratory for Agricultural Chemistry, Uppsala, Sweden, using the method of Novikov (1971).

Serum T-4 Concentration

The serum was analysed for T-4 concentration using the laboratory facilities of Dr. F. L. Lorscheider, Faculty of Medicine, University of Calgary. The Ames Tetralute ^{125}I kit (Ames Division, Miles Laboratories Inc., Elkhart, Ind.) was utilized following the method described by Braverman et al. (1971).

Serum T-3 Concentration

Serum T-3 concentrations were also determined in Dr. Lorscheider's laboratory using the Mallincrodt RIA-Mat Circulating T-3 ^{125}I kit (Mallincrodt Nuclear, St. Louis, Mo.). The procedure used in this kit is based on the methods of Larsen (1972), Lieblich and Utiger (1972), and Surks et al. (1972).

Thyroid Radioiodine Half-Life

The rats were injected ip. with 3 μCi of ^{131}I (Charles E. Frosst and Co., Toronto, Ont.) in 1 ml of physiological saline. Exactly 48 hours later, each rat was anesthetized in a dessicator containing a cotton swab soaked in halothane (Fluothane, Ayerst Laboratories, St. Laurent, Que.). Halothane was used rather than sodium pentobarbitol or diethyl ether because the rats regained consciousness more quickly.

Once ansthetized, the rats had their thyroid radioactivity measured using the apparatus shown in Figure 2. This apparatus consisted of a 10 mm lead plate containing a 1 cm detection aperture focused over a 1 inch sodium iodide detection crystal. The aperture was filled with a plastic casting resin to prevent contamination of the crystal and to eliminate differences in counting rate due to differences in downward pressure on the rat. Detection was accomplished using a Nuclear Chicago Model DS 202 detector (Nuclear Chicago, Des Plaines, Ill.) in conjunction with a rate metre and a Nuclear Chicago Model 8725 analyser-scaler. The thyroid region was positioned over the aperture until maximum counts registered on the rate metre and a 30 second count was then taken with the scaler. Four separate counts were taken on each rat over a 72 hour period, the last three being adjusted for radioactive decay. A regression was run on time versus counts (calculated as a percent of administered dose) to determine the rate of thyroid radioiodine loss. This figure was converted to thyroid radioiodine half-life using the equations of Wang and Willis (1965).

^{131}I - 48 Hour Thyroid Uptake

This was determined by taking the initial count in the thyroid radioiodine half-life determination and expressing it as a percent of administered dose.

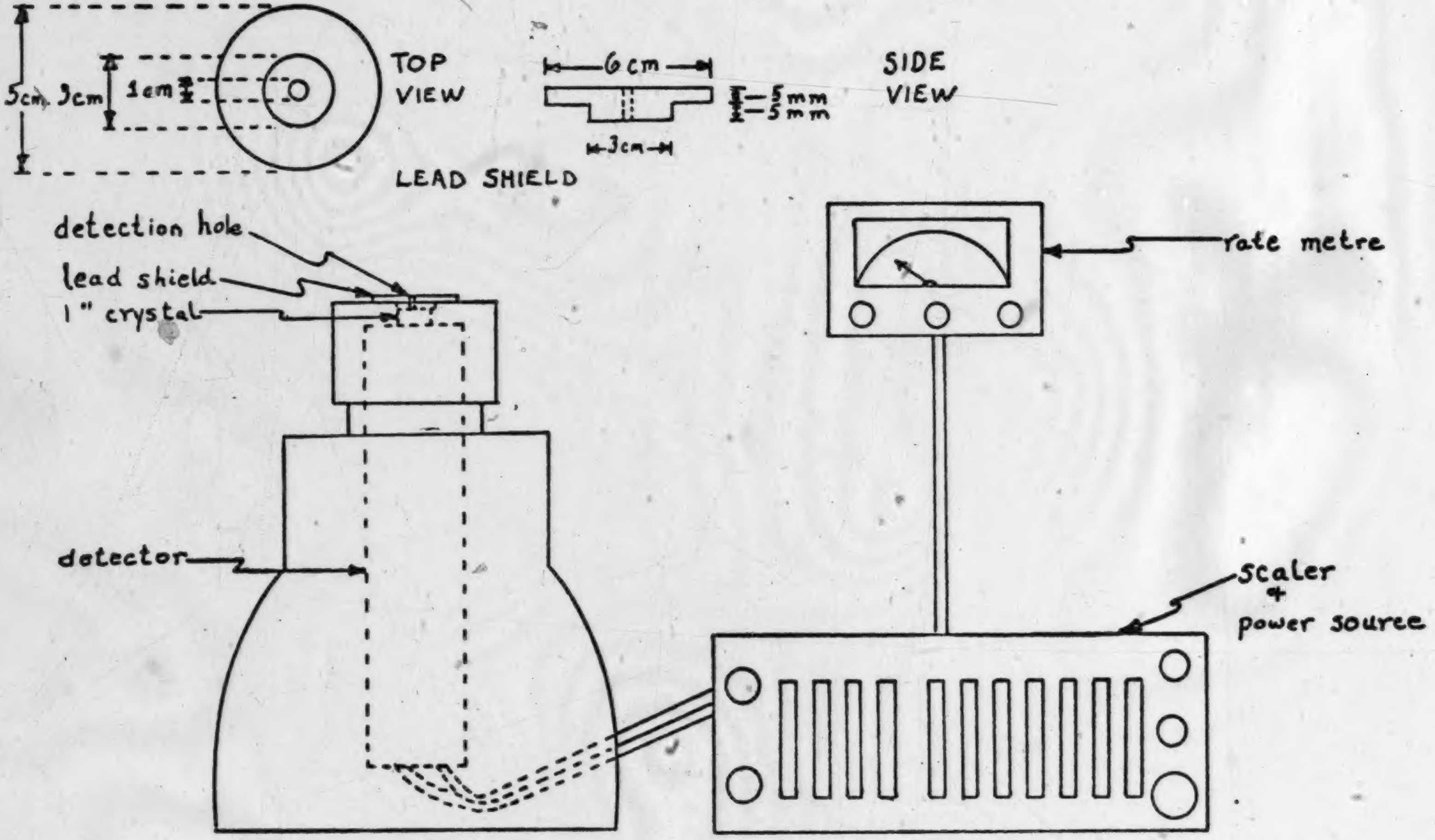

FIGURE 2. Apparatus for Taking Thyroid Counts in the Live Rat

Milk Composition

Milk samples were analysed for fat with a Milko-tester (A/SN Foss Electric, Hillerod, Denmark), solids-not-fat by the bead test (Golding, 1964), and protein according to AOAC (1965) methods.

STATISTICAL METHODS

Analyses of variance were computed using the University of Alberta Computing Centre Programme C.S. 2384 (Weingardt, 1973). Where significant differences were found, multiple comparisons of means were performed ($P < 0.05$) using Duncan's New Multiple Range Test (Steele and Torrie, 1960). Significance is noted by marking means not significant from each other with the same superscript. Significance is only noted for means of single classification groups.

In computing the analyses of variance, there were three basic types of difference tables according to the data available.

(i) Values given for rats and periods - weight gains

(ii) Values given for rats only - single measurement data including thyroid weights, radioiodine uptakes, T-4's, T-3's, thyroid radioiodine half-lives, and any transformations done on the parameters.

(iii) Values given for cages and periods - feed, milk, and iodine consumption.

In the weight gains and single measurement data, the rat variation was used as an estimate of error in Trials I and II, but in Trial III the cage variation could be used. The use of rat variation in Trials I and II was justified by the Trail III data, since the rat variation was not appreciably greater than the cage variation. For the consumption data, the three factor and above interactions were pooled to obtain an estimate of error in Trials I and II but the cage variation was again used in Trial III.

There were two areas of missing data which had to be estimated in this study. In Trial II, one whole block was missing (STORE-High I-Male) and it was approximated using a multiway classification missing data formula (Steele and Torrie, 1960) before the analysis of variance was run. In Trial III a total of six rats died during the determination of thyroid radioiodine half-life and their values were estimated from their cage means.

PRELIMINARY INVESTIGATIONS

A preliminary 7 week trial was run in the winter of 1974 using six male and six female Sprague-Dawley weanling rats divided among six cages. Each cage was assigned to one of two treatment groups, with two cages of females and one cage of males in the RSM group and two cages of males and one cage of females in the FABA group. All rats received the high energy basal ration (Table 1).

The rats were also fed ad libitum either FABA-milk or RSM-milk (according to their treatment group) as their only source of liquid. The FABA-milk was from a cow receiving faba beans at 20.5% of the concentrate ration and the RSM-milk was from a cow receiving RSM at 8.6% of the concentrate ration. All milk was fed as skim milk and treated and stored as in the other trials.

Throughout the trial, weekly cage data was kept on weight gains and feed and milk consumption. Sacrificing, bleeding, PBI analysis, and 24-hour ^{125}I uptake were done as in Trial I except that thyroids were counted as part of a tracheal section.

The results indicated no significant treatment differences for the weight gain or consumption data, but there was a definite sex effect since the males ate more feed and grew faster, than the females. There were no significant sex or treatment differences for PBI nor sex differences for thyroid radioiodine uptake. The RSM group however had a significantly higher thyroid radioiodine uptake than the FABA group.

Although this trial was too small to draw any accurate conclusions, the radioiodine uptake data showed that further investigation into the goitrogenic properties of RSM-milk was justified. This preliminary trial appears to implicate iodine deficiency because of the elevated radioiodine uptakes, but goitrogens in the RSM-milk could not be ruled out at this stage.

RESULTS

To facilitate interpretation, the results for each experimental parameter shall be presented on a trial basis. All significant differences refer to the 5% level ($P < 0.05$) and do not infer that significance occurs only at this level.

TRIAL I

Trial I will be treated as two separate two-way classifications, with the sexes compared across both liquids for the five week data, and the two time periods (2 weeks and 5 weeks) compared across the liquid groups for the female data.

Values for Feed and Milk Iodine Concentrations

1. Feed iodine concentration. The feed contained 0.04 mg I/kg as analysed by Dr. Karin Thente.

2. Milk iodine concentrations. The milk iodine concentrations for each sample collected are found in Table 4. These values were all elevated because of the high iodine salt consumed, but the RSM-milk showed significantly lower iodine concentrations than the FABA-milk.

Sex-Liquid Classification

The results of the sex-liquid classification are found in Tables 5 and 6.

1. Feed, milk, and iodine intake. The FABA group

TABLE 4

TRIAL I - MILK IODINE CONCENTRATIONS

Date	Milking	RSM (μg/100 ml)	FABA (μg/100 ml)
April 19	A. M.	46.9	139.4
April 25	A. M.	84.6	147.3
May 3	P. M.	107.1	139.8
May 8	A. M.	125.9	162.8
May 12	P. M.	115.5	160.1
May 17	P. M.	98.5	174.5
May 21	A. M.	82.1	98.4
AVERAGE TEST		94.4^{b}	146.0^{a}

TABLE 5

TRIAL I - LIQUID-SEX GROUP* MEANS FOR TOTAL FEED, MILK AND IODINE CONSUMPTION AND TOTAL WEIGHT GAINS

Comparison	Group	Feed Intake		Milk Intake		Iodine Intake		Weight Gains (g/rat/ Week)
		(g/cage/ Week)	(g/rat/ Day)	(ml/cage/ Week)	(ml rat/ Day)	(μg/cage/ Week)	(μg/rat/ Day)	
1. Liquids (8 rats per group)	RSM	372^{a}	13.3^{a}	816^{a}	29.1^{a}	816^{b}	29.1^{b}	31.9^{a}
	FABA	378^{a}	13.5^{a}	839^{a}	30.0^{a}	1323^{a}	47.3^{a}	33.9^{a}
2. Sexes (8 rats per group)	F	319^{b}	11.4^{b}	808^{b}	28.9^{b}	1049^{b}	37.5^{b}	26.5^{b}
	M	430^{a}	15.4^{a}	847^{a}	30.3^{a}	1089^{a}	38.9^{a}	39.3^{a}
3. Liquid-sex (4 rats per group)	RSM-F	319	11.4	783	28.0	797	28.5	25.5
	FABA-F	319	11.4	832	29.7	1301	46.5	27.5
	RSM-M	424	15.1	848	30.3	835	29.8	38.4
	FABA-M	436	15.6	846	30.2	1344	48.0	40.3

*Five week trial.

TABLE 6

TRIAL I - LIQUID-SEX GROUP[*] MEANS FOR THYROID GLAND CHARACTERISTICS AND SERUM PBI

Comparison	Group	Thyroid Weight Absolute (mg/rat)	Thyroid Weight Relative (mg/100 g body wt)	Thyroid 24-hr. ^{125}I Uptake Absolute (% of Dose)	Thyroid 24-hr. ^{125}I Uptake Relative (% of Dose/ 10 mg thyroid wt)	PBI (µg/100 ml)
1. Liquids (8 rats per group)	RSM	10.9^a	5.3^a	2.23^a	2.05^a	2.0^a
	FABA	12.1^a	5.7^a	1.86^a	1.55^b	2.4^a
2. Sexes (8 rats per group)	F	10.8^a	6.1^a	2.01^a	1.87^a	1.8^b
	M	12.2^a	4.9^b	2.08^a	1.72^a	2.6^a
3. Liquid-Sex (4 rats per group)	RSM-F	10.2	5.9	2.04	1.99	1.7
	FABA-F	11.3	6.3	1.99	1.75	2.0
	RSM-M	11.6	4.7	2.43	2.10	2.3
	FABA-M	12.9	5.1	1.73	1.35	2.8

[*] Five week trial.

consumed significantly more iodine because of the higher milk iodine concentrations, but there were no significant differences in feed or milk consumption. In the sex comparison, the males consumed more feed, milk, and iodine than the females.

2. Weight gains. The FABA males and females gained faster than their respective RSM groups, but the overall difference between the liquid groups wasn't significant. The sex comparison reveals a significantly faster weight gain for the males.

3. Thyroid weights (absolute and relative to body weight). There were no significant differences in thyroid weights (absolute or relative) between the liquid groups, although the RSM groups had smaller thyroid weights in both sexes. In the sex comparison, the females had higher thyroid weights, but only significantly so when calculated relative to body weight.

4. 24-hour radioiodine uptake (absolute and relative to thyroid weight). The radioiodine uptakes were all low (less than 3% of the administered dose) presumably because of the high iodine concentrations in the milk. The RSM group had a significantly higher radioiodine uptake than the FABA group when the uptake was taken relative to thyroid weight. No other significant differences in absolute or relative radioiodine uptake occurred in the liquids or sex comparisons.

5. Serum protein-bound iodine concentrations. The RSM group had lower PBI values in both sexes, but the overall difference was not quite significant (P = 0.058). In the sex comparison, the females had significantly lower PBI's than the males.

Time Period-Liquid Classification

Since the animals in the 2 week trial may have been at a different point on their growth curve than those in the 5 week trial, no attempt was made to compare the feed, milk, or iodine consumption data or growth rates. The results of the time period-liquid classification for the single measurement data are found in Table 7 and are summarized below.

1. Thyroid weights (absolute and relative to body weight). There were no differences in either the time period or liquid comparisons for the absolute thyroid weights or thyroid weights relative to body weight.

2. 24-hour radioiodine uptakes (absolute and relative to thyroid weight). The RSM group showed consistently higher radioiodine uptakes, the difference being significant when uptake was calculated relative to thyroid weight. There were no significant differences in the 2 week- 5 week comparison regardless of how radioiodine uptake was calculated.

3. Serum protein-bound iodine concentrations. The RSM groups in both time periods had lower PBI values than their respective FABA groups, but the overall difference

TABLE 7

TRIAL I - LIQUID-TIME PERIOD[*] GROUP MEANS FOR THYROID GLAND CHARACTERISTICS AND SERUM PBI

Comparison	Group	Thyroid Weight		Thyroid 24-hr. ^{125}I Uptake		PBI (µg/100 ml)
		Absolute (mg/rat)	Relative (mg/100 g body wt)	Absolute (% of Dose)	Relative (% of Dose/ 10 mg thyroid wt)	
1. Liquids (8 rats per group)	RSM	10.9^{a}	6.3^{a}	2.12^{a}	1.92^{a}	1.2^{a}
	FABA	11.5^{a}	6.4^{a}	1.63^{a}	1.42^{b}	1.5^{a}
2. Time periods (8 rats per group)	5 week	10.8^{a}	6.1^{a}	2.01^{a}	1.87^{a}	1.8^{a}
	2 week	11.7^{a}	6.7^{a}	1.74^{a}	1.48^{a}	0.8^{b}
3. Liquid-Time Period (4 rats per group)	RSM - 5 week	10.2	5.9	2.04	1.99	1.7
	FABA- 5 week	11.3	6.3	1.99	1.75	2.0
	RSM - 2 week	11.7	6.7	2.20	1.80	0.7
	FABA- 2 week	11.8	6.6	1.27	1.10	0.9

[*] Groups contain only female rats.

wasn't significant. The rats on the 2 week trial had significantly lower PBI values than the rats on the 5 week trial.

TRIAL II

Trial II involved comparisons of five liquids (RSM, FABA, BRON, WATER, STORE), two feed iodine levels (Low I, High I), and both sexes. For purposes of presentation, liberal use is made of tables, line graphs, and histograms, and only brief summaries are included in the text.

The tables are used to show group means for the various parameters measured. Tables 9 and 10 give the overall liquid, feed, and sex group means; Tables 11 and 12 show the liquid-feed group means; and Tables 13 and 14 present the liquid-sex group means. If more detail is required for verification purposes, the individual cage means are given in Appendices A-1 and A-2.

The line graphs (Figures 3-6) have been used to show the weekly liquid group means for the feed, milk, and iodine intakes and the weight gains. The same group means for the single measurement data are found in the histograms (Figures 7-13).

1. Feed iodine concentrations. The iodine concentrations in the Low I and High I feeds as analyzed by Dr. Karin Thente, were 0.04 mg I/kg and 2.0 mg I/kg respectively.

2. Milk iodine concentrations. The iodine concentrations for each milk sample taken are in Table 8. The STORE-milk had a significantly higher iodine concentration than the other milks, and the FABA-milk had a significantly higher concentration than the RSM- or BRON-milks.

3. Milk production and composition. The total milk production and the average milk composition values for each cow over the period of this trial are found in Appendix C-2. The RSM-fed cows had considerably higher milk production than the FABA-fed cows, which in turn had higher production than the BRON- fed cows. The only major difference in the composition averages was the depressed % fat of the RSM group.

4. Feed consumption. The WATER group ate significantly more feed than the milk groups (Figure 3) but no significant differences in feed consumption occurred between the milk groups. The males ate significantly more feed than the females, but in the feeds comparison there was no significant difference in feed consumption.

5. Milk Consumption. The FABA group drank significantly less milk than the other milk groups (Figure 4) and this is accounted for mainly in the two FABA-Low I groups (Table 11). The High I feed group drank significantly more milk than the Low I feed group but there was no difference between the sex groups.

TABLE 8

TRIAL II - MILK IODINE CONCENTRATIONS

Date	Milking	RSM Milk (µg/100 ml)	FABA Milk (µg/100 ml)	BRON Milk (µg/100 ml)	Store Milk (µg/100 ml)
Aug. 20	A. M.	6.8	17.1	7.4	17.4
Aug. 23	A. M.	8.5	21.6	7.4	25.3
Aug. 26	P. M.	11.9	16.7	11.7	6.8
Aug. 29	A. M.	9.5	22.2	10.7	-
Sept. 2	A. M.	7.6	7.6	5.1	16.1
Sept. 5	P. M.	5.1	4.5	4.5	36.3
Sept. 8	P. M.	6.9	11.1	4.6	17.8
Sept. 13	A. M.	5.7	15.1	3.0	23.0
Sept. 16	P. M.	7.3	8.1	4.6	27.7
Sept. 20	P. M.	7.6	10.1	1.7	25.7
Sept. 24	P. M.	7.8	6.7	5.9	35.5
Sept. 28	P. M.	10.4	11.1	6.6	28.4
AVERAGE TEST		7.9^c	12.7^b	6.1^c	23.6^a

TABLE 9

TRIAL II - GROUP MEANS FOR TOTAL FEED, MILK AND IODINE CONSUMPTION AND TOTAL WEIGHT GAINS

Comparison	Group	Feed Intake		Milk Intake		Iodine Intake		Weight Gains
		(g/cage/ Week)	(g/rat/ Day)	(ml/cage/ Week)	(ml/rat/ Day)	(µg/cage/ Week)	(µg/rat/ Day)	(g/rat/week)
1. Liquids (16 rats per group)	RSM	433^{b}	15.5^{b}	936^{a}	33.4^{a}	516^{c}	18.4^{c}	28.8^{a}
	FABA	436^{b}	15.6^{b}	866^{b}	30.9^{b}	520^{c}	18.6^{c}	27.4^{a}
	BRON	441^{b}	15.8^{b}	926^{a}	33.1^{a}	493^{c}	17.6^{c}	26.8^{a}
	WATER	[illegible]08^{a}	18.1^{a}	0^{c}	0.0^{c}	578^{b}	20.6^{b}	21.4^{b}
	STORE	407^{b}	14.5^{b}	919^{a}	32.8^{a}	628^{a}	22.4^{a}	27.5^{a}
2. Feeds (40 rats per group)	LOW I	440^{a}	15.7^{a}	709^{b*}	25.3^{b}	103^{a}	3.7^{a}	26.4^{a}
	HIGH I	451^{a}	16.1^{a}	750^{a*}	26.8^{a}	991^{b}	35.4^{b}	26.3^{a}
3. Sexes (40 rats per group)	F	365^{a}	13.0^{a}	733^{a*}	26.2^{a}	452^{a}	16.1^{a}	18.2^{b}
	M	526^{b}	18.8^{b}	726^{a*}	25.9^{a}	642^{b}	22.9^{b}	34.5^{a}

* Average includes rats in water groups. Significance remains unchanged when water groups omitted.

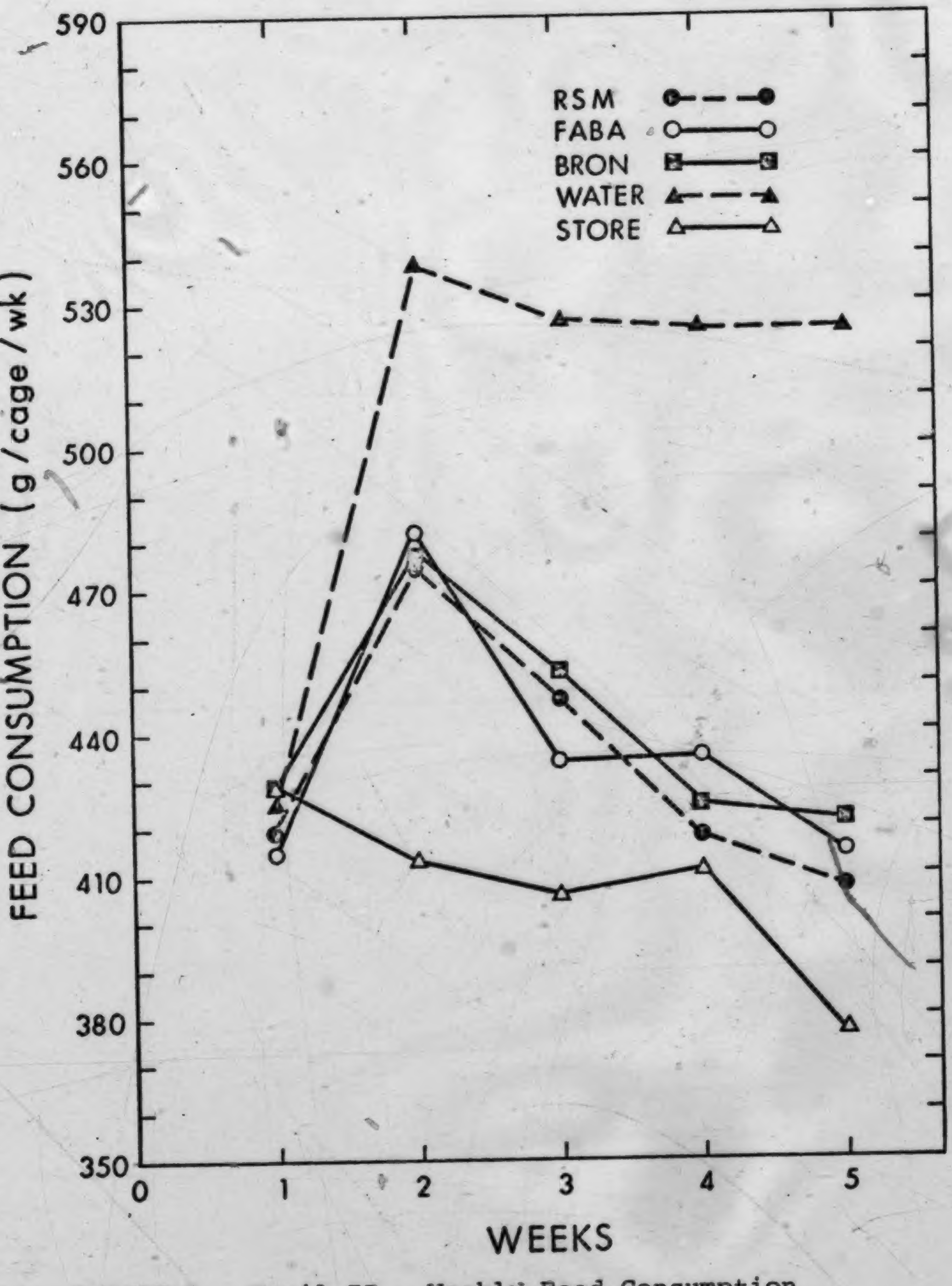

FIGURE 3. Trail II - Weekly Feed Consumption

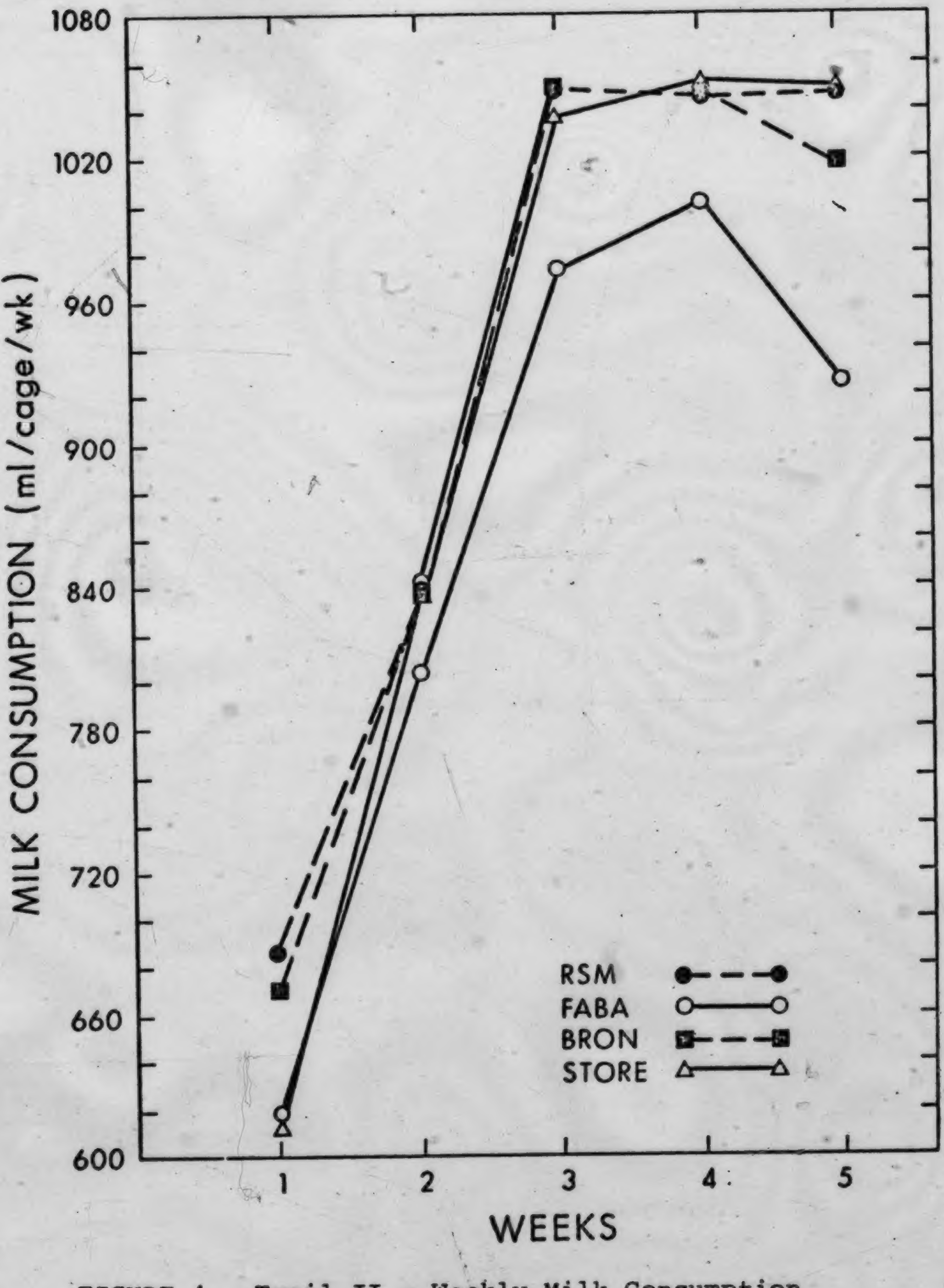

FIGURE 4. Trail II - Weekly Milk Consumption

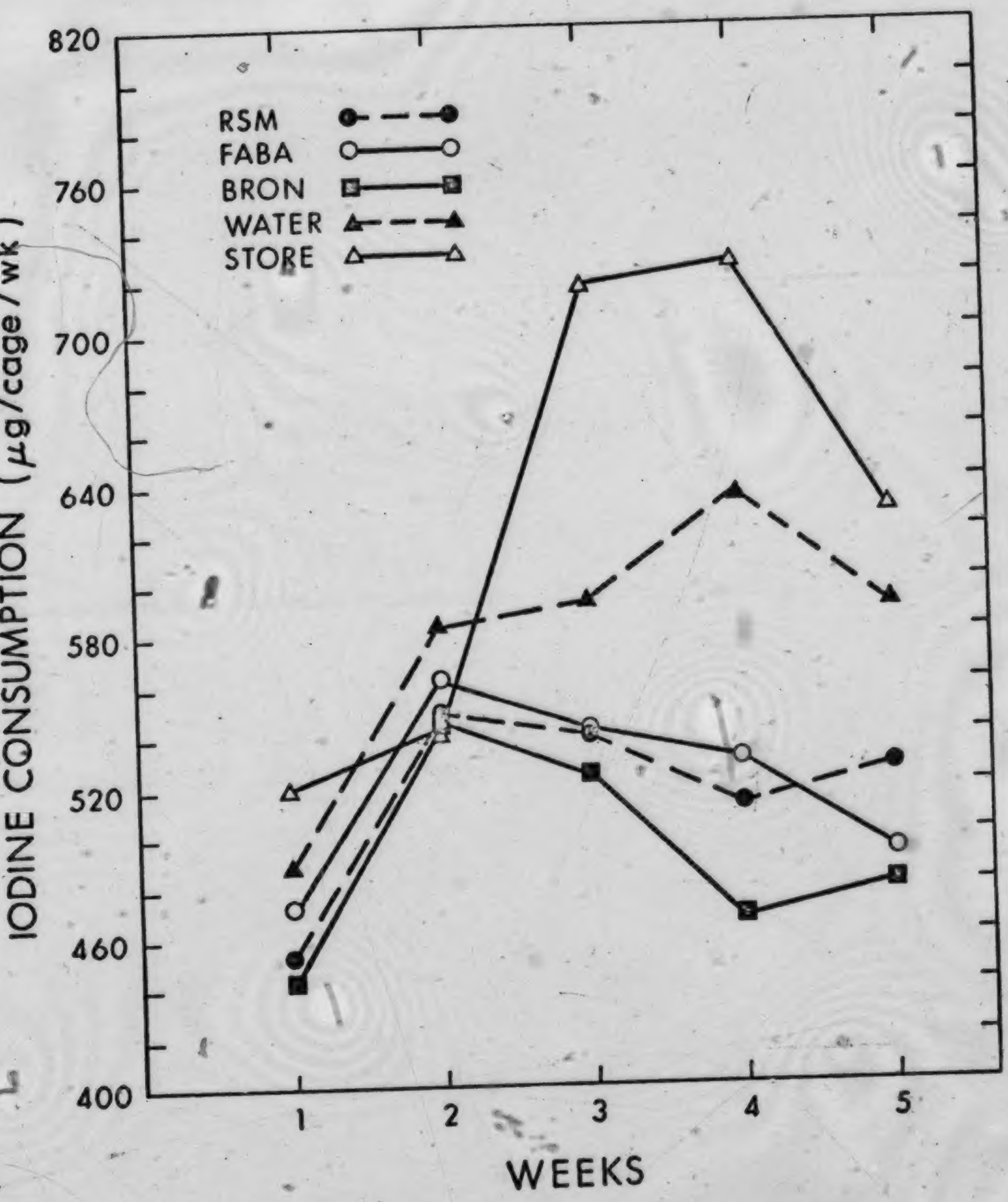

FIGURE 5. Trial II - Weekly Iodine Consumption

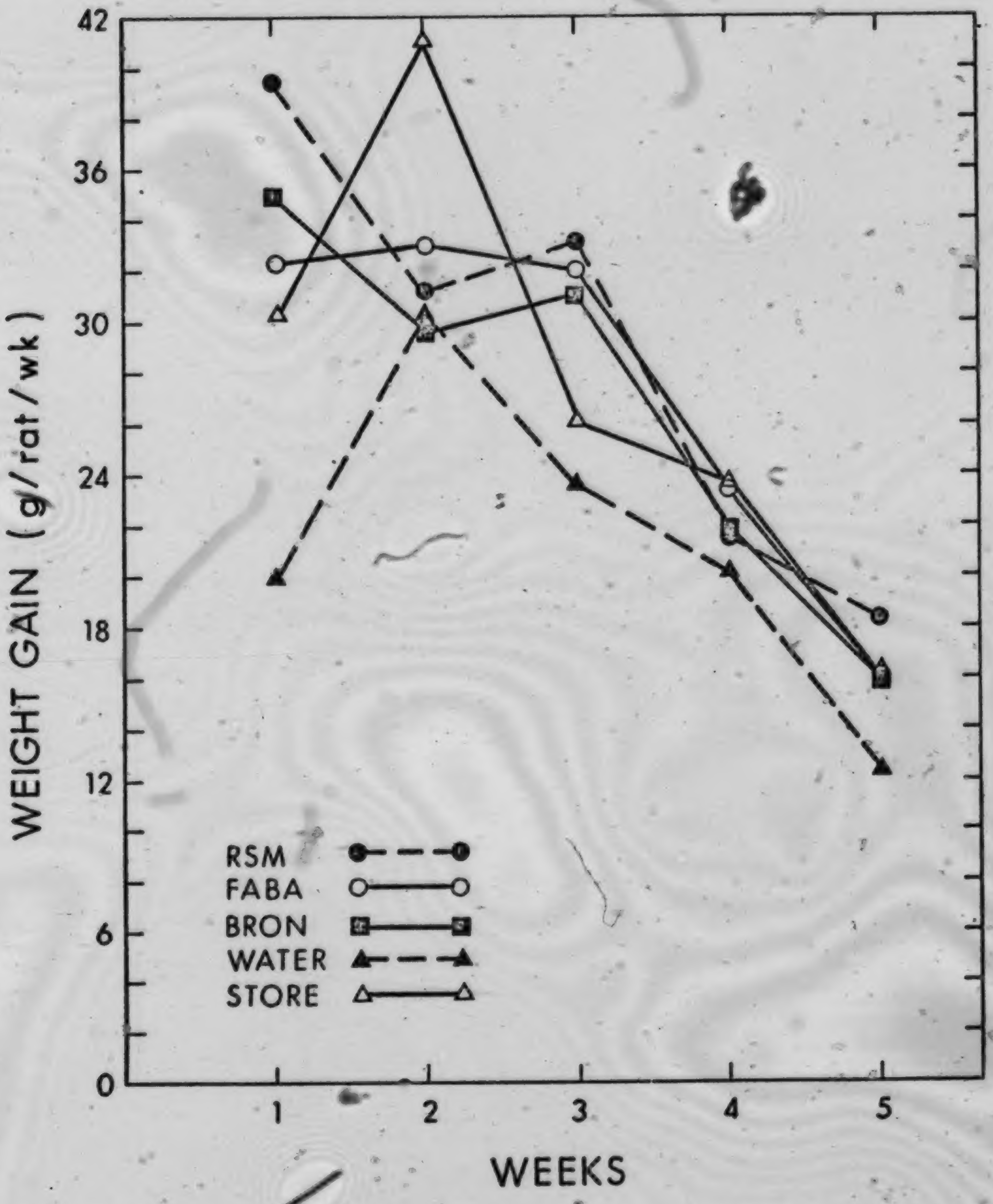

FIGURE 6. Trial II - Weekly Weight Gains

TABLE 10

TRIAL II - GROUP MEANS FOR THYROID GLAND CHARACTERISTICS AND SERUM THYROID HORMONE VALUES

Comparison	Group	Thyroid Weight		Thyroid 24-hr. ^{125}I Uptake		Serum Thyroid Hormone		
		Absolute (mg/rat)	Relative (mg/100 g body wt)	Absolute (% of Dose)	Relative (% of Dose/ 10 mg Thyroid Wt)	T-4 (μg/100 ml)	T-3 (ng/100 ml)	T-3/T-4 (ng/100 ml/μg/ 100 ml)
1. Liquids (16 rats per group)	RSM	12.5^{a}	4.6^{b}	10.42^{c}	8.18^{c}	4.2^{b}	93.8^{a}	22.1^{ab}
	FABA	10.3^{b}	3.9^{c}	11.61^{bc}	11.68^{b}	4.5^{ab}	90.8^{a}	20.6^{bc}
	BRON	10.9^{b}	4.2^{c}	13.67^{b}	12.35^{b}	4.4^{ab}	97.0^{a}	22.0^{ab}
	WATER	11.1^{b}	5.5^{a}	27.76^{a}	24.25^{a}	4.3^{b}	96.5^{a}	23.8^{a}
	STORE	10.1^{b}	3.8^{c}	4.97^{d}	4.75^{d}	4.9^{a}	86.3^{a}	17.9^{c}
2. Feeds (40 rats per group)	LOW I	11.3^{a}	4.8^{a}	23.14^{a}	20.39^{a}	4.5^{a}	99.1^{a}	22.8^{a}
	HIGH I	10.6^{b}	3.9^{b}	4.24^{b}	4.09^{b}	4.5^{a}	86.6^{b}	19.7^{b}
3. Sexes (40 rats per group)	F	9.7^{b}	4.7^{a}	13.50^{a}	12.88^{a}	4.0^{b}	90.4^{a}	22.7^{a}
	M	12.3^{a}	4.1^{b}	13.88^{a}	11.60^{a}	4.9^{a}	95.4^{a}	19.8^{b}

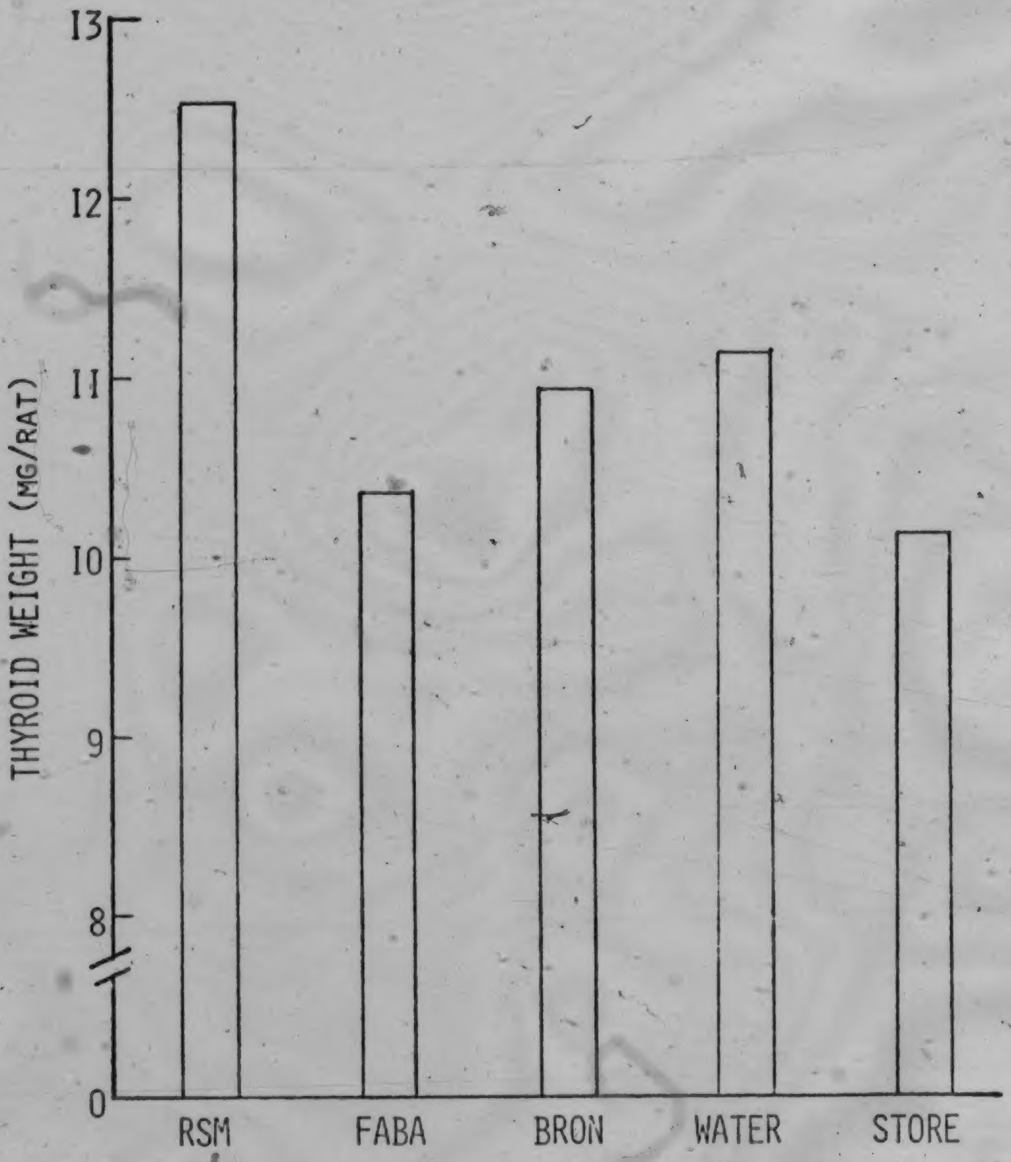

FIGURE 7. TRIAL II - Liquid Group Means for Thyroid Weights

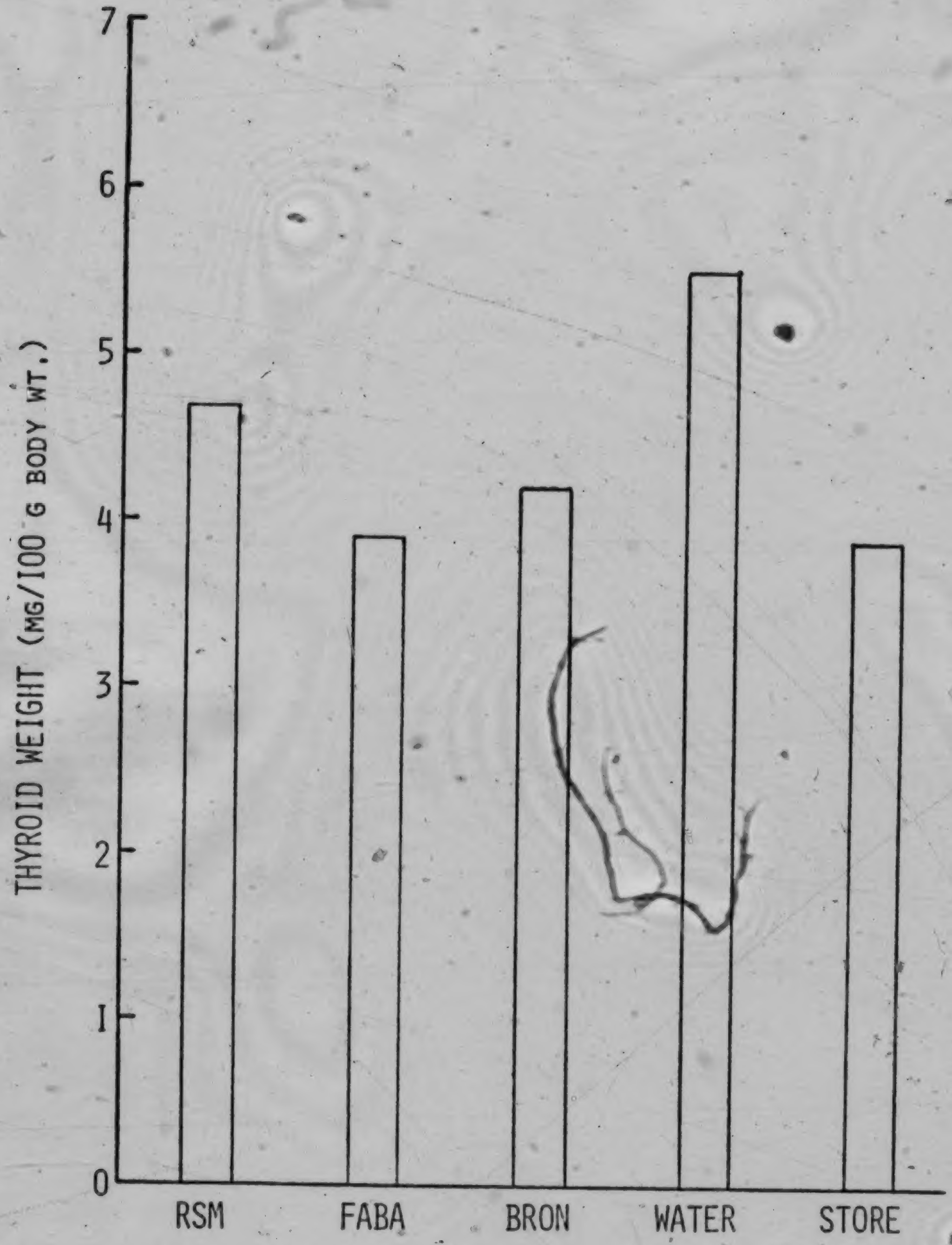

FIGURE 8. TRIAL II - Liquid Group Means for Relative Thyroid Weights

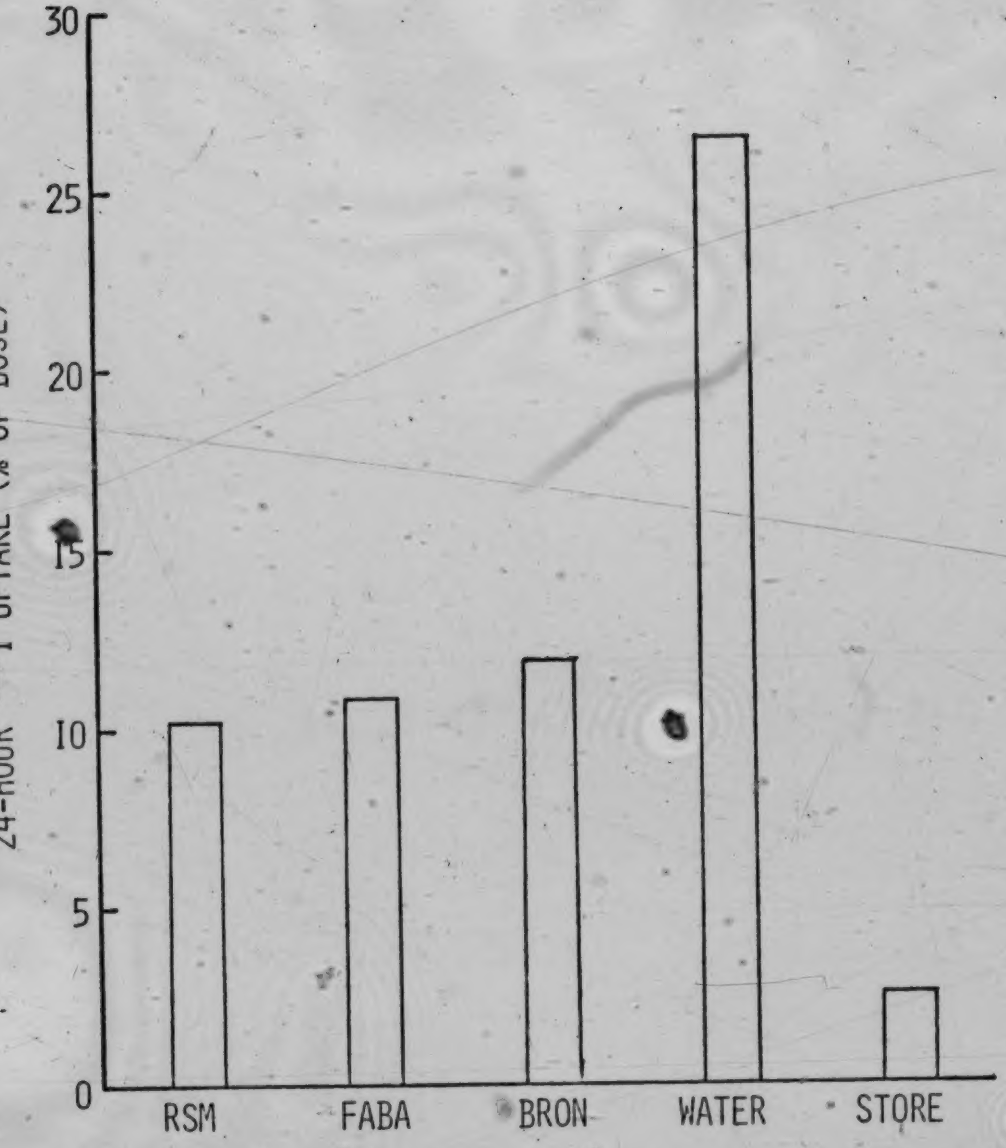

FIGURE 9. TRIAL II - Liquid Group Means for Thyroid Radioiodine Uptake

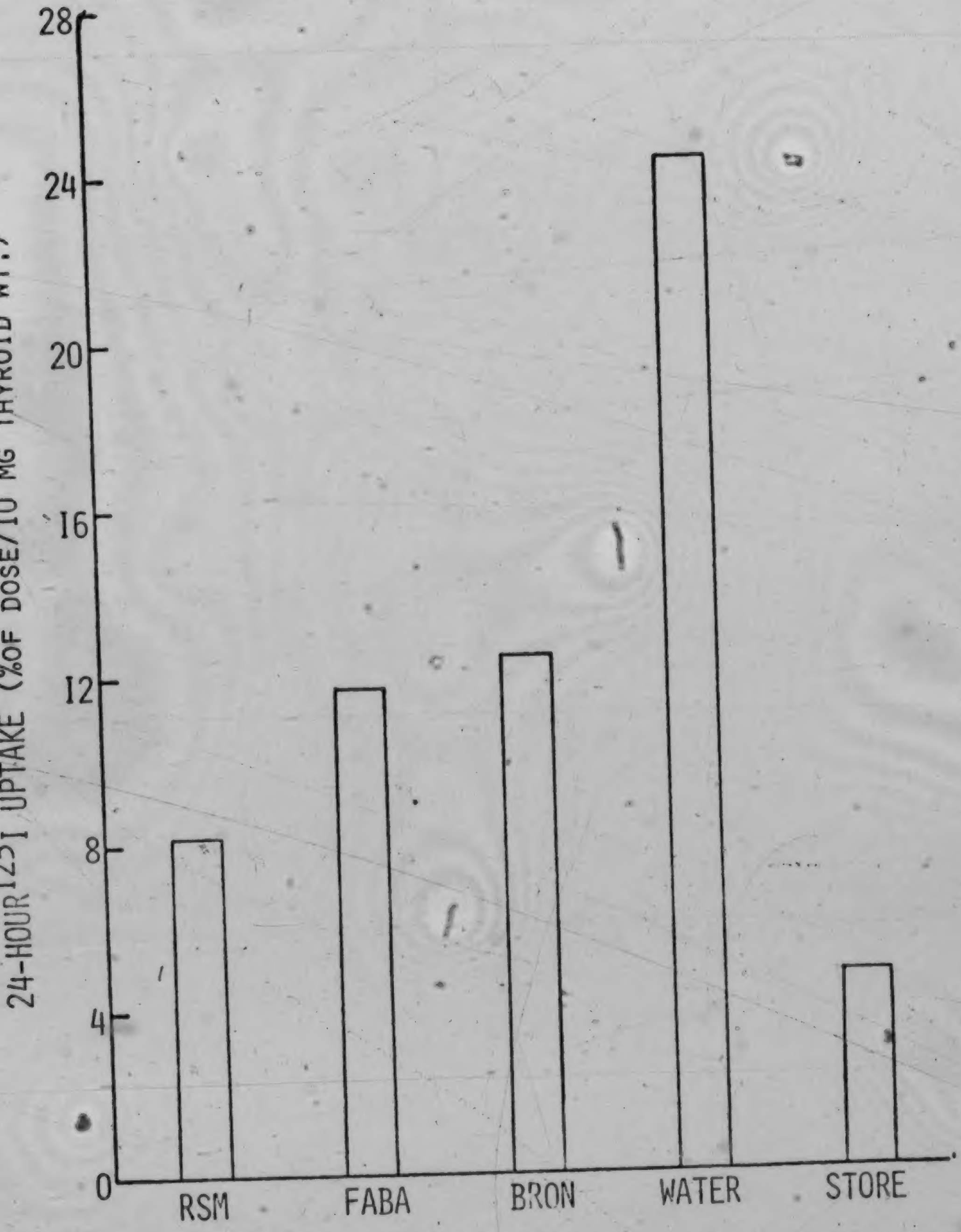

FIGURE 10. TRIAL II - Liquid Group Means for Relative Thyroid Radioiodine Uptake

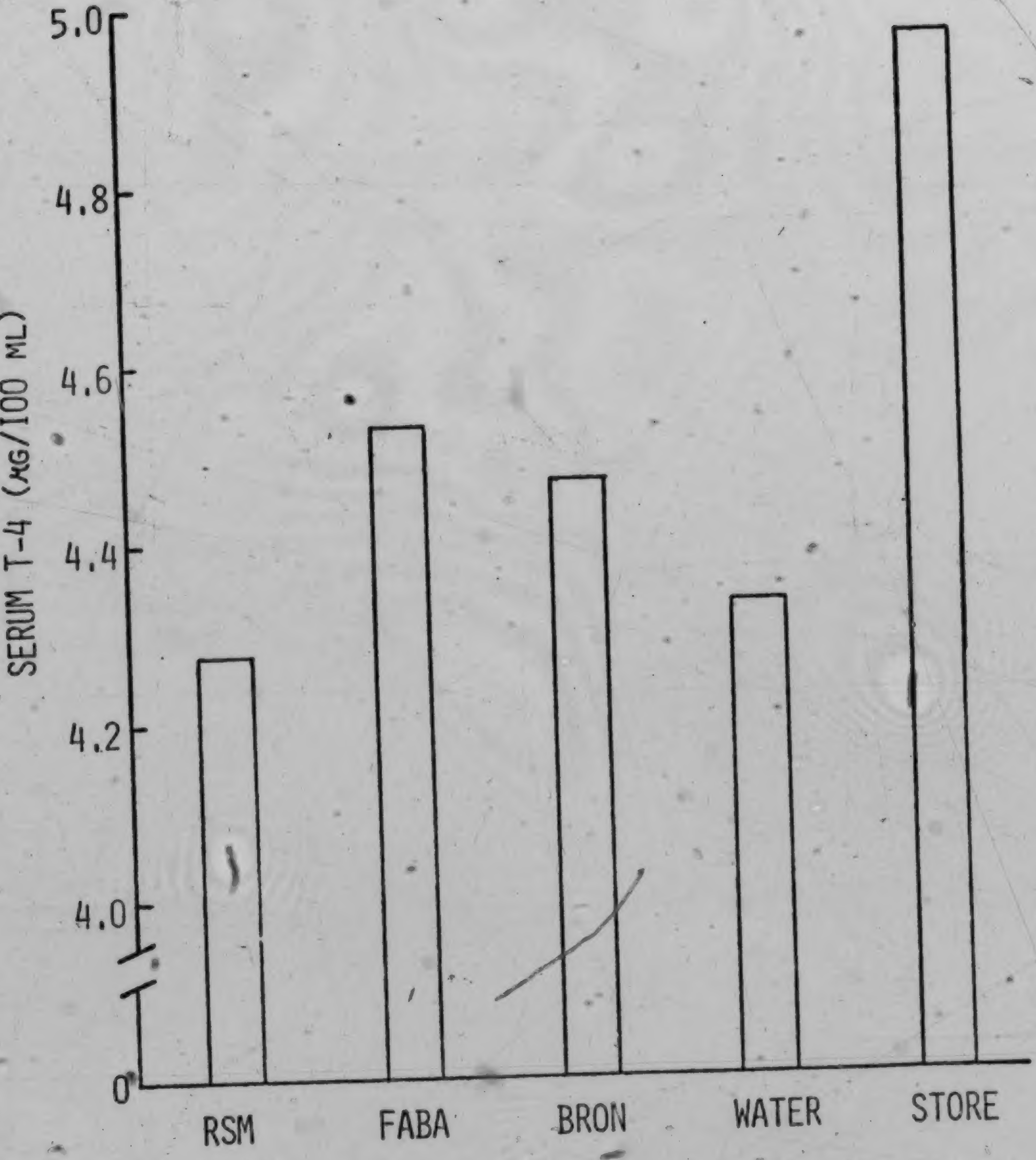

FIGURE 11. TRIAL II - Liquid Group Means for Serum T-4

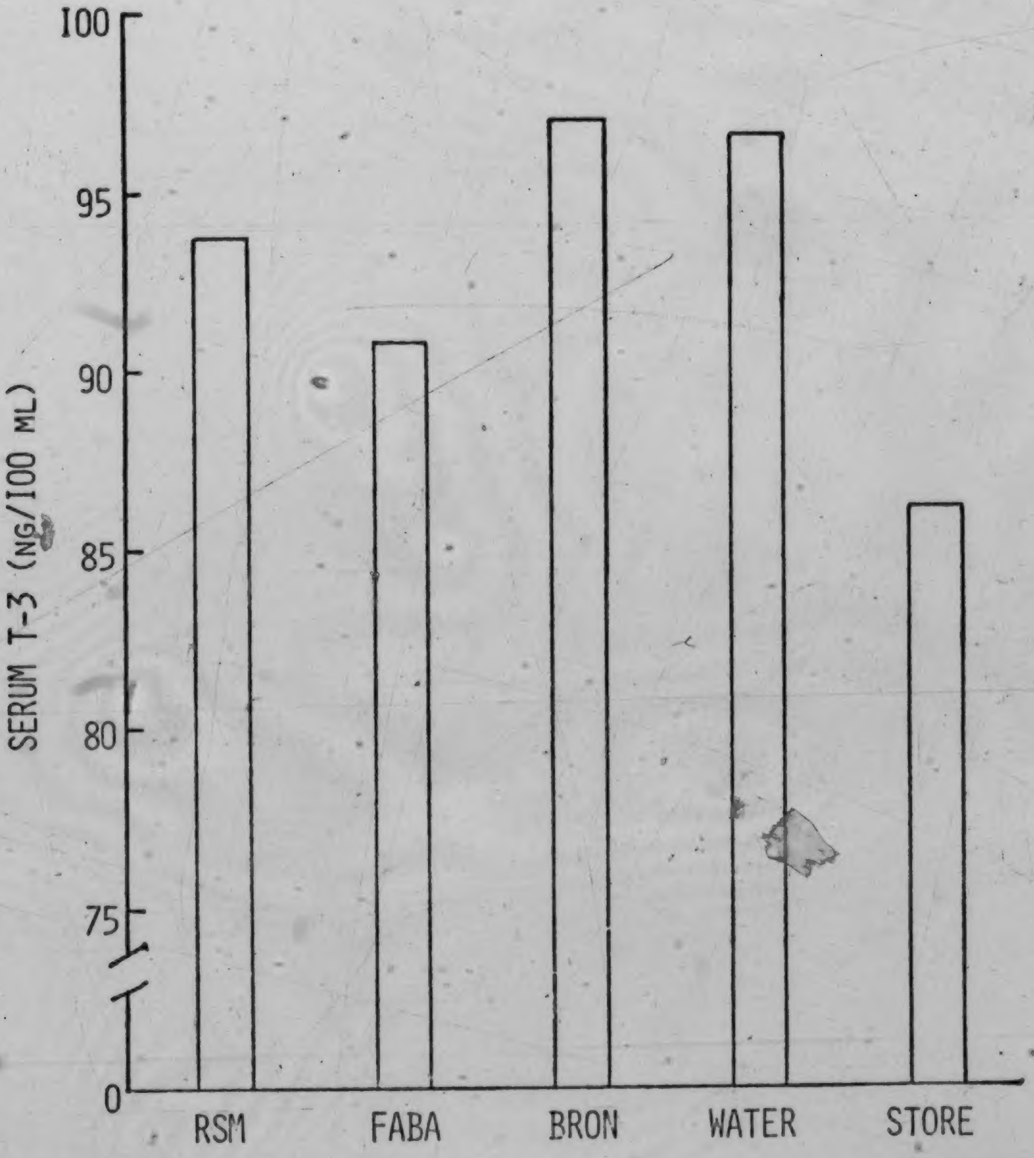

FIGURE 12. TRIAL II - Liquid Group Means for Serum T-3

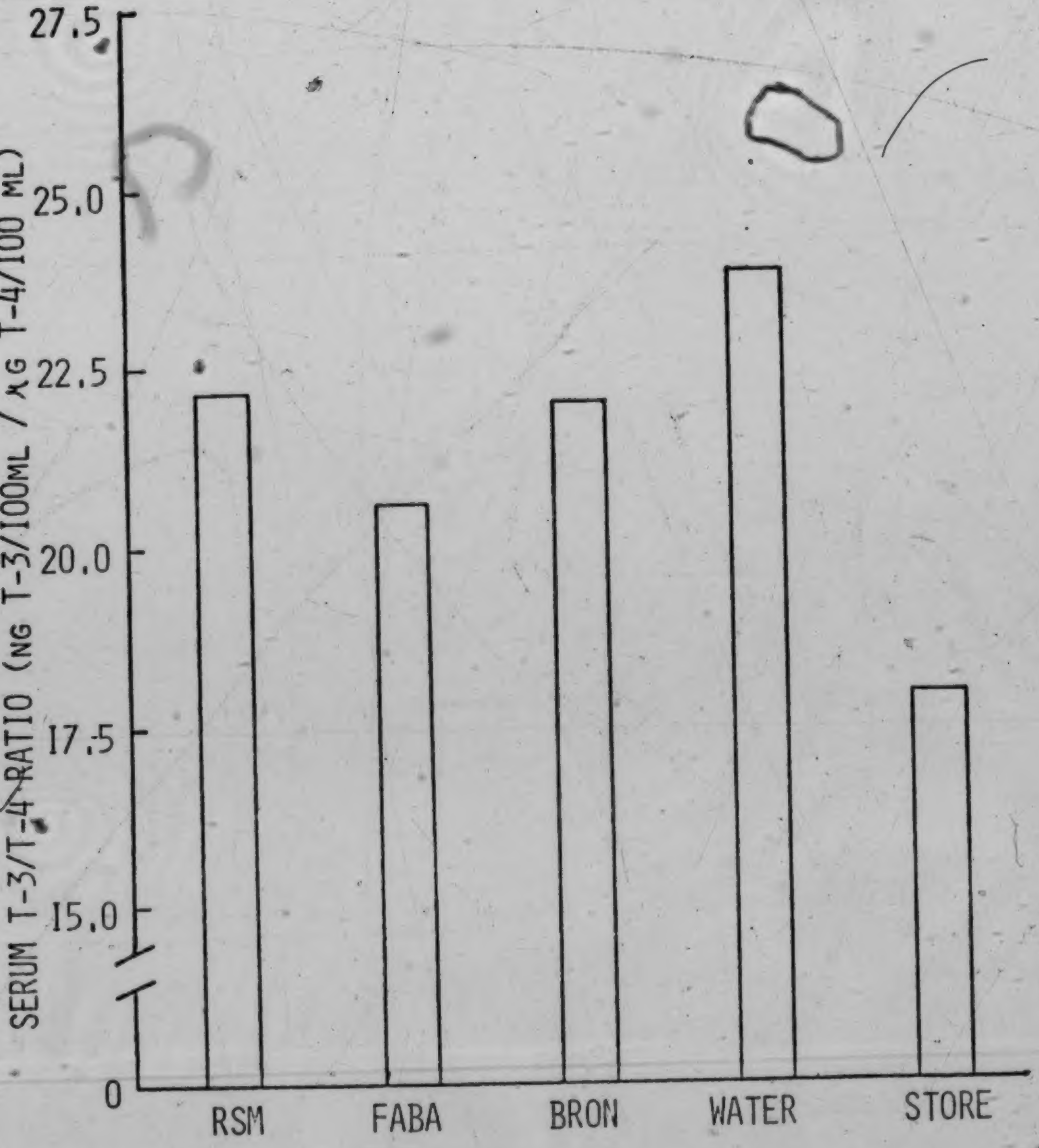

FIGURE 13. TRIAL II - Liquid Group Means for Serum T-3/T-4 Ratio

TABLE 11

TRIAL II - LIQUID-FEED GROUP MEANS FOR FEED, MILK AND IODINE CONSUMPTION AND TOTAL WEIGHT GAINS

Group		Feed Intake		Milk Intake		Iodine Intake		Weight Gains
Liquid	Feed	(g/cage/ week)	(g/rat/ day)	(ml/cage/ week)	(ml/rat/ day)	(µg/cage/ week)	(µg/rat/ day)	(g/rat/ week)
RSM	LOW I	427	15.3	934	33.4	87	3.1	29.8
FABA	LOW I	463	16.5	794	28.4	120	4.3	30.4
BRON	LOW I	451	16.1	914	32.6	74	2.6	31.3
WATER	LOW I	447	16.0	0	0.0	18	0.6	11.0
STORE	LOW I	411	14.7	904	32.3	214	7.6	29.4
RSM	HIGH I	439	15.7	938	33.5	945	33.8	27.8
FABA	HIGH I	410	14.6	938	33.5	920	32.9	24.4
BRON	HIGH I	432	15.4	938	33.5	911	32.5	22.2
WATER	HIGH I	569	20.3	0	0.0	1139	40.7	31.7
STORE	HIGH I	404	14.4	934	33.4	1042	37.2	25.5

Each group contains 8 rats.

TABLE 12

TRIAL II - LIQUID-FEED GROUP MEANS FOR THYROID GLAND CHARACTERISTICS AND SERUM THYROID HORMONE VALUES

Group		Thyroid Weight		Thyroid 24-hr. ^{125}I Uptake		Serum	Thyroid	Hormone
Liquid	Feed	Absolute (mg/rat)	Relative (mg/100 g body wt)	Absolute (% of Dose)	Relative (% of Dose/ 10 mg Thyroid wt)	T-4 (µg/100 ml)	T-3 (ng/100 ml)	T-3/T-4 (ng/100 ml/µg 100 ml)
RSM	LOW I	13.0	5.0	16.66	12.78	4.3	91.0	20.9
FABA	LOW I	9.8	3.7	18.03	18.34	4.6	94.8	20.8
BRON	LOW I	11.4	4.3	23.80	21.23	4.5	102.3	22.9
WATER	LOW I	11.7	6.9	49.07	42.20	3.6	111.3	31.2
STORE	LOW I	10.8	4.3	8.13	7.41	5.4	96.1	18.4
RSM	HIGH I	12.0	4.2	4.19	3.58	4.2	96.7	23.3
FABA	HIGH I	10.9	4.0	5.18	5.02	4.4	86.7	20.4
BRON	HIGH I	10.4	4.0	3.55	3.47	4.3	91.6	21.1
WATER	HIGH I	10.6	4.1	6.46	6.30	4.9	81.7	16.4
STORE	HIGH I	9.4	3.4	1.80	2.08	4.5	76.5	17.4

Each group contains 8 rats.

TABLE 13

TRIAL II - LIQUID-SEX GROUP MEANS FOR FEED, MILK, AND IODINE CONSUMPTION AND TOTAL WEIGHT GAINS

Group		Feed Intake		Milk Intake		Iodine Intake		Weight Gains
Liquid	Sex	(g/cage/ week)	(g/rat/ day)	(ml/cage/ week)	(ml/rat/ day)	(µg/cage/ week)	(µg/rat/ day)	(g/rat/ week)
RSM	F	340	12.1	934	33.4	434	15.5	19.6
FABA	F	347	12.4	894	31.9	417	14.9	17.8
BRON	F	340	12.1	922	32.9	392	14.0	17.9
WATER	F	485	17.3	0	0.0	494	17.6	18.7
STORE	F	312	11.1	915	32.7	525	18.8	16.9
RSM	M	526	18.8	938	33.5	599	21.4	37.9
FABA	M	526	18.8	837	29.9	623	22.3	37.0
BRON	M	542	19.4	930	33.2	594	21.2	35.6
WATER	M	531	19.0	0	0.0	662	23.6	24.1
STORE	M	503	18.0	915	32.7	730	26.1	38.0

Each group contains 8 rats.

TABLE 14

TRIAL II - LIQUID-SEX GROUP MEANS FOR THYROID GLAND CHARACTERISTICS AND SERUM THYROID HORMONE VALUES

Group		Thyroid Weight		Thyroid 24-hr. ^{125}I Uptake		Serum	Thyroid	Hormone
Liquid	Sex	Absolute (mg/rat)	Relative (mg/100 g body wt)	Absolute (% of Dose)	Relative (% of Dose/ 10 mg Thyroid wt)	T-4 (µg/100 ml)	T-3 (ng/100 ml)	T-3/T-4 (ng/100 ml/µg 100 ml)
RSM	F	10.8	5.0	9.68	8.58	4.1	94.1	22.8
FABA	F	8.3	3.9	10.04	12.58	4.0	87.2	22.3
BRON	F	10.1	4.9	14.47	13.61	4.2	98.0	23.7
WATER	F	10.6	5.6	28.34	24.42	4.0	91.5	24.5
STORE	F	8.7	4.1	4.96	5.22	4.0	81.1	20.3
RSM	M	14.2	4.2	11.17	7.78	4.4	93.6	21.4
FABA	M	12.4	3.8	13.18	10.78	5.0	94.3	18.9
BRON	M	11.7	3.4	12.88	11.09	4.7	96.0	20.3
WATER	M	11.6	5.4	27.19	24.08	4.6	101.6	23.1
STORE	M	11.5	3.6	4.98	4.28	5.8	91.5	15.5

Each group contains 8 rats.

6. Iodine consumption. The STORE-milk group consumed significantly more iodine than the other groups because of its high milk iodine concentration (Figure 5). The WATER group consumed significantly more iodine than the RSM, FABA, or BRON groups because of the higher consumption of the High I feed (Table 11). There were no significant differences in iodine consumption within the RSM-, FABA-, or BRON-milk groups. The High I feed group showed significantly higher iodine intake than the Low I feed group. The males consumed significantly more iodine than the females because of their higher feed consumption (Table 13).

7. Weight gains. The WATER group gained weight significantly slower than any of the milk groups, but there were no differences in weight gains within the milk groups. As seen in Figure 6, the RSM group had the highest weight gain during the first week of the experiment but this difference did not continue for the rest of the trial. There was no difference between the High I and Low I feed groups, but the males gained weight significantly faster than the females.

8. Thyroid weights (absolute). The RSM groups had significantly larger thyroids than the other liquid groups (Figure 7) and it is important to note that this difference persisted over both levels of feed iodine (Table 12). There were also significant sex and feed differences with the males

and Low I feed group having the largest thyroids in each classification (Table 10).

9. Thyroid weights (relative to body weight). The WATER group had significantly larger relative thyroid weights than the milk groups due mainly to the WATER-Low I group (Table 12). The RSM group had significantly larger relative thyroid weights than the other milk groups (Figure 8) and Table 12 shows that this difference again persisted on both levels of feed iodine. In the sex comparison the females had significantly larger relative thyroid weights, as did the Low I group in the feed comparison.

10. 24-hour radioiodine uptake (absolute). The WATER group had a significantly greater radioiodine uptake, and the STORE group had a significantly smaller radioiodine uptake than the other groups (Figure 9). The RSM group had the second lowest radioiodine uptake, being significantly lower than the BRON group due to the differences in the Low I feed groups (Table 12). There were no differences in the sex comparison, but the Low I feed group had significantly higher absolute uptake than the High I feed group (Table 10).

11. 24-hour radioiodine uptake (relative to thyroid weight). In the liquid groups (Figure 10) the order was the same and the significance similar to the absolute thyroid uptake of radioiodine except that the RSM group was significantly lower than the FABA- and BRON-milk groups. Once again the RSM and BRON difference was found mainly in the Low I feed groups, but the RSM was considerably lower in relative

radioiodine uptake than the FABA group over both feed iodine levels (Table 12). For the sex and feed comparisons, the order and significance was the same as for the absolute radioiodine uptake.

12. Serum T-4 concentrations. In the liquids comparison, the WATER and RSM groups had the lowest serum T-4 concentrations, but were only significantly different from the STORE group. No significant difference existed in the feed iodine comparison, but in the sex comparison, the males had a significantly higher mean serum T-4 concentration than the females.

13. Serum T-3 concentrations. There were no significant differences in serum T-3 concentrations in the liquids comparisons, but the RSM, BRON, and WATER serum T-3 concentrations were higher than the STORE and FABA groups (Figure 12). The Low I feed group resulted in a significantly elevated mean serum T-3 concentration, but there was no difference between sexes.

14. Serum T-3/T-4 ratios. The STORE group had a significantly lower serum T-3/T-4 ratio than all of the liquids except the FABA group, and the FABA group had a significantly lower ratio than the WATER group. There were no significant differences between the RSM, FABA, and BRON groups. In the feeds comparison, the Low I group had a significantly elevated serum T-3/T-4 ratio and in the sex comparison, the females had a significantly higher serum T-3/T-4 value.

TRIAL III

Trial III involved the comparison of seven liquids (RSM-H, FABA-H, RSM-M, FABA-M, RSM-L, FABA-L, WATER), two feed iodine levels (Low I, High I), using only female rats. Females were chosen because they appeared to show thyroid inhibition more readily than males in the previous trials.

The tables presented for the Trial III rat data are as follows: Table 16 gives the overall means for all parameters measured in the RSM and FABA groups, regardless of the cow salt mix; Tables 17-19 give the liquid and feed group means; and Tables 20-22 give the liquid-feed group means. If further detail is required, Appendices B-1 to B-3 contain the cage means for this data.

The line graphs (Figures 14-17) show the weekly liquid group means for the feed, milk, and iodine consumption and weight gain data. The histograms (Figures 18-25) show the liquid group means for the single measurement data.

1. Feed iodine concentrations. The iodine concentrations in the Low I and High I feeds as measured by Dr. Karin Thente, were 0.15 mg I/kg and 0.28 mg I/kg respectively.

2. Milk iodine concentrations. The milk iodine concentration for each sample taken is found in Table 15. For all but the L salt mix, the Faba-milk was significantly higher in iodine than the RSM-milk. It can also be seen that the iodine content of the milk was directly related to the iodine concentration of the salt mix fed.

TABLE 15

TRIAL III - MILK IODINE CONCENTRATIONS

Date	Milking	High I Salt RSM (µg/ 100 ml)	High I Salt FABA (µg/ 100 ml)	Medium I Salt RSM (µg/ 100 ml)	Medium I Salt FABA (µg/ 100 ml)	Low I Salt RSM (µg/ 100 ml)	Low I Salt FABA (µg/ 100 ml)
Nov. 7	A. M.	12.2	18.4	3.3	14.3	1.7	3.3
Nov. 11	A. M.	9.8	14.4	3.0	7.2	0.4	2.5
Nov. 14	A. M.	8.2	20.0	4.5	11.8	0.3	2.5
Nov. 18	A. M.	7.5	19.4	2.9	12.4	0.7	0.2
Nov. 21	A. M.	10.0	41.2	2.6	9.9	0.9	0.6
Nov. 25	A. M.	10.6	42.6	3.2	16.1	0.0	0.8
Nov. 28	A. M.	14.2	27.4	7.4	15.6	1.3	2.7
Dec. 2	A. M.	10.2	12.3	3.6	9.5	0.9	1.4
Dec. 5	A. M.	9.3	12.6	4.5	11.9	1.9	1.9
Dec. 9	A. M.	8.7	13.6	2.7	11.8	0.4	1.4
Average Test		10.1^b	22.2^a	3.8^c	12.1^b	0.9^c	1.7^c

3. Milk production and composition. The total milk production and average milk composition values for each cow over the period of this trial are found in Appendix C-4. The RSM-fed cows generally produced more milk than the FABA-fed cows, but these differences were likely the result of cow allocation problems due to the small herd size rather than an effect of the grain rations. There were no significant differences in % fat, % protein, or % SNF between any of the groups. Overall it does not appear that the low iodine intake of RSM-L and FABA-L cow groups affected their milk composition (other than iodine) or production during this trial.

4. Feed consumption. Figure 14 and Table 17 show that the WATER group ate significantly more feed than any of the milk groups, but there were no significant differences in feed consumption between the milk groups. The iodine concentration of the feeds caused no difference in the consumption of the feeds (Table 17).

5. Milk consumption. Figure 15 reveals no significant differences in milk consumption for any of the milk-fed groups. This observation is reinforced by the statistical data in Table 17. There was no significant difference in milk consumption between the two feed groups.

6. Iodine consumption. The iodine intake of a group was related directly to the iodine concentration of the liquid it consumed, the only exception being the WATER

TABLE 16

TRIAL III - OVERALL MEANS FOR RSM AND FABA GROUPS

(EACH GROUP REPRESENTS: 72 RATS)

Parameter	Units	RSM	FABA
Feed Intake	(g/cage/week)	298^a	303^a
Milk Intake	(ml/cage/week)	708^a	709^a
Iodine Intake	(μg/cage/week)	100^b	153^a
Weight Gains	(g/rat/week)	23.2^a	23.7^a
Thyroid Weight (Absolute)	(mg/rat)	11.0^a	9.9^b
Thyroid Weight (Relative)	(mg/100 g body wt)	5.3^a	4.6^b
48-hr ^{131}I Uptake (Absolute)	(% of Dose)	36.51^a	29.97^b
48-hr ^{131}I Uptake (Relative)	(% of Dose)	33.75^a	30.55^a
T-4	(μg/100 ml)	3.6^a	3.6^a
T-3	(g/100 ml)	90.0^a	83.1^a
T-3/T-4	(ng/100 ml/ g/ 100 ml)	25.6^a	24.8^a
Thyroid ^{131}I Half-life	(days)	1.72^b	2.12^a

TABLE 17

TRIAL III - GROUP MEANS FOR TOTAL FEED, MILK, AND IODINE CONSUMPTION, AND TOTAL WEIGHT GAINS

Comparison	Group	Feed Intake		Milk Intake		Iodine Intake		Weight Gains
		(g/rat/ week)	(g/rat/ day)	(ml/cage/ day)	(ml/rat/ day)	(µg/cage/ week)	(µg/rat/ day)	(g/rat/week)
1. Liquids (24 rats per group)	RSM-H	306^b	10.9^b	709^a	25.3^a	140^c	5.0^c	23.3^a
	FABA-H	300^b	10.7^b	717^a	25.6^a	229^a	8.2^a	23.4^a
	RSM-M	291^b	10.4^b	710^a	25.4^a	90^d	3.2^d	22.9^a
	FABA-M	303^b	10.8^b	697^a	24.9^a	151^b	5.4^b	23.5^a
	RSM-L	298^b	10.6^b	703^a	25.1^a	71^f	2.5^f	23.3^a
	FABA-L	308^b	11.0^b	712^a	25.4^a	78^e	2.8^e	24.1^a
	WATER	389^a	13.9^a	0^b	0^b	83^e	3.0^e	20.9^a
2. Feeds (84 rats per group)	LOW I	313^a	11.2^a	604^{a*}	21.6^a	100^b	3.6^b	22.9^a
	HIGH I	313^a	11.2^a	610^{a*}	21.8^a	141^a	5.0^a	23.2^a

*Average includes rats in water groups. , Significance remains unchanged when water groups omitted.

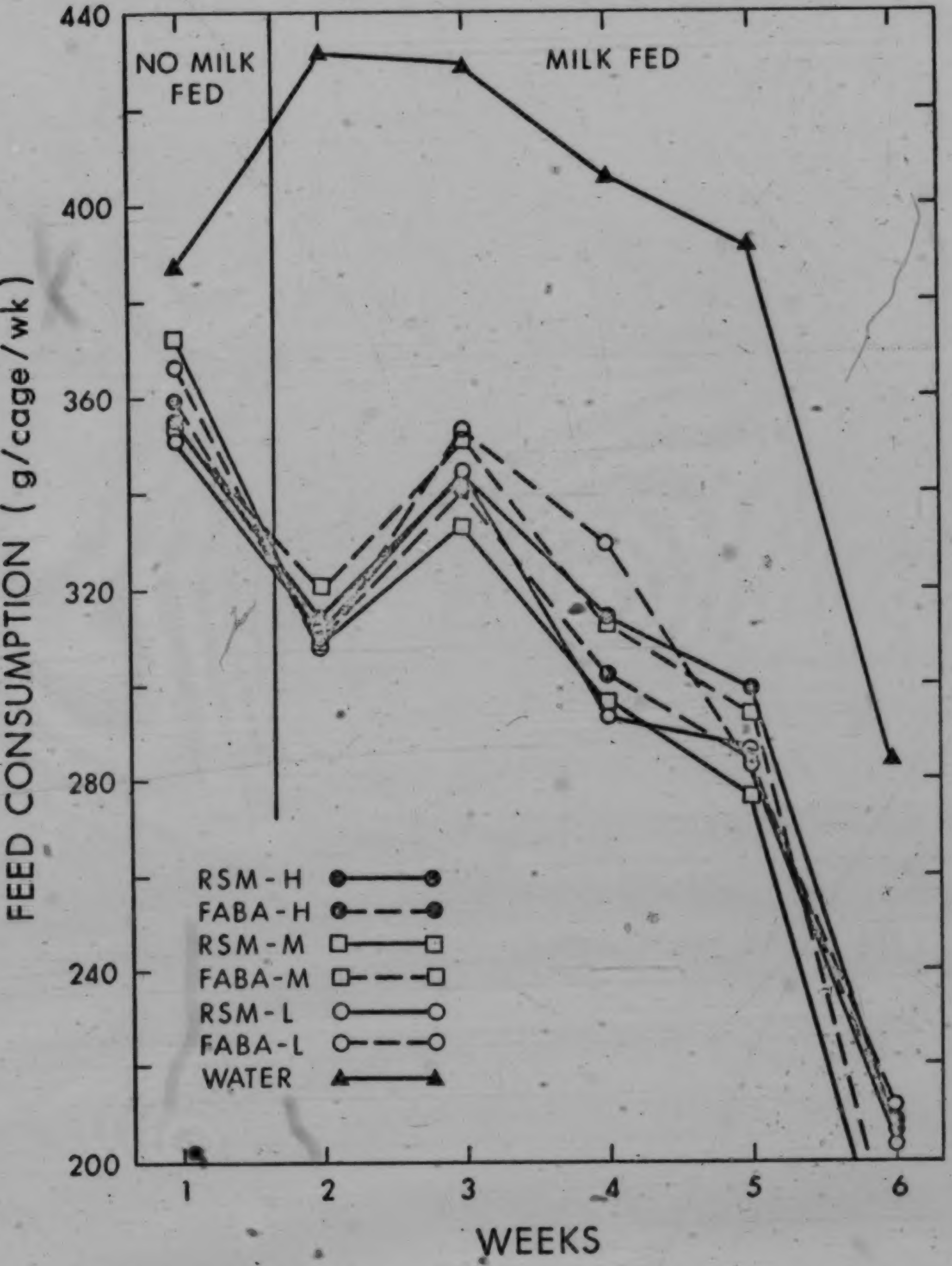

FIGURE 14. Trial III - Weekly Feed Consumption

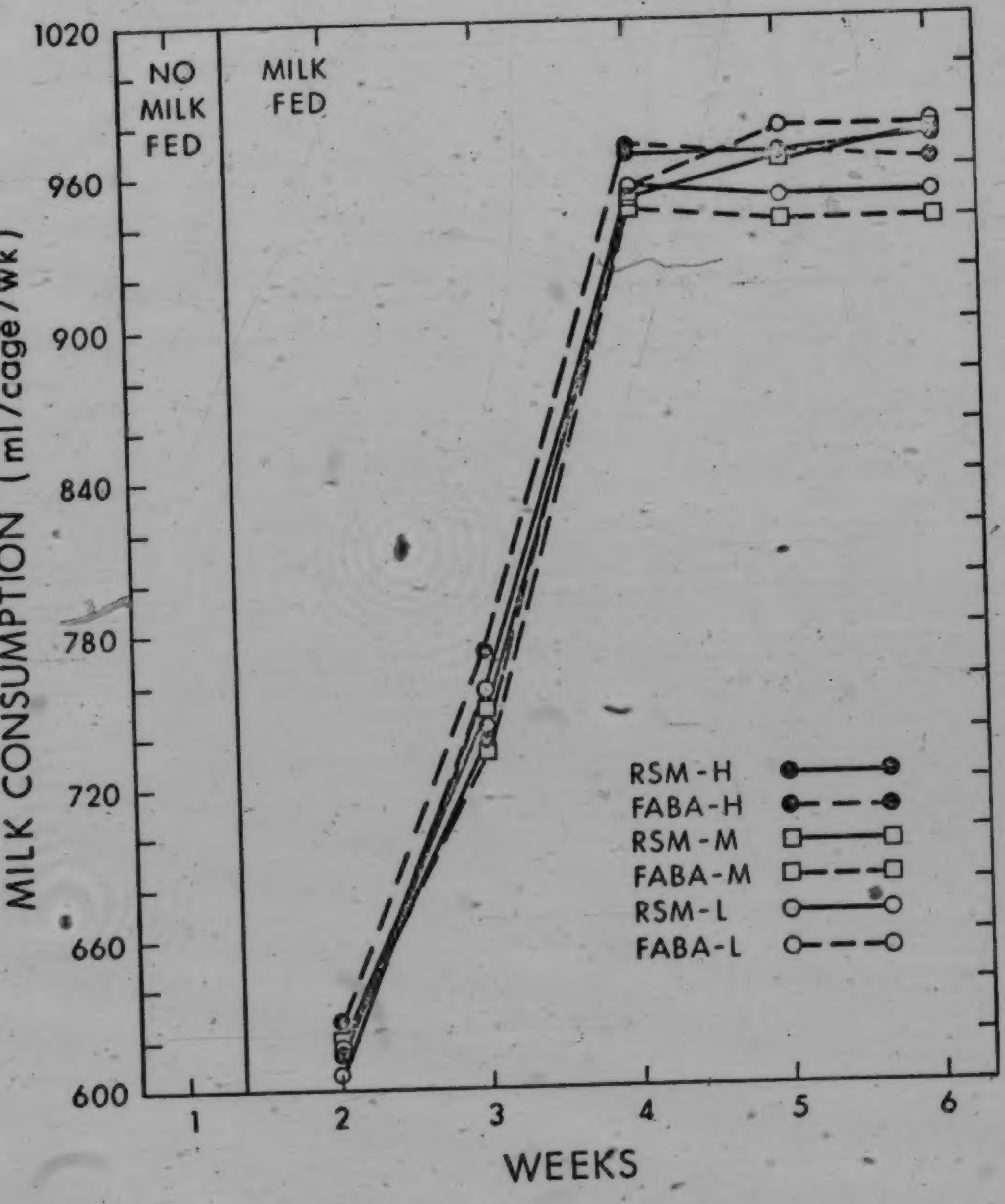

FIGURE 15. Trial III - Weekly Milk Consumption

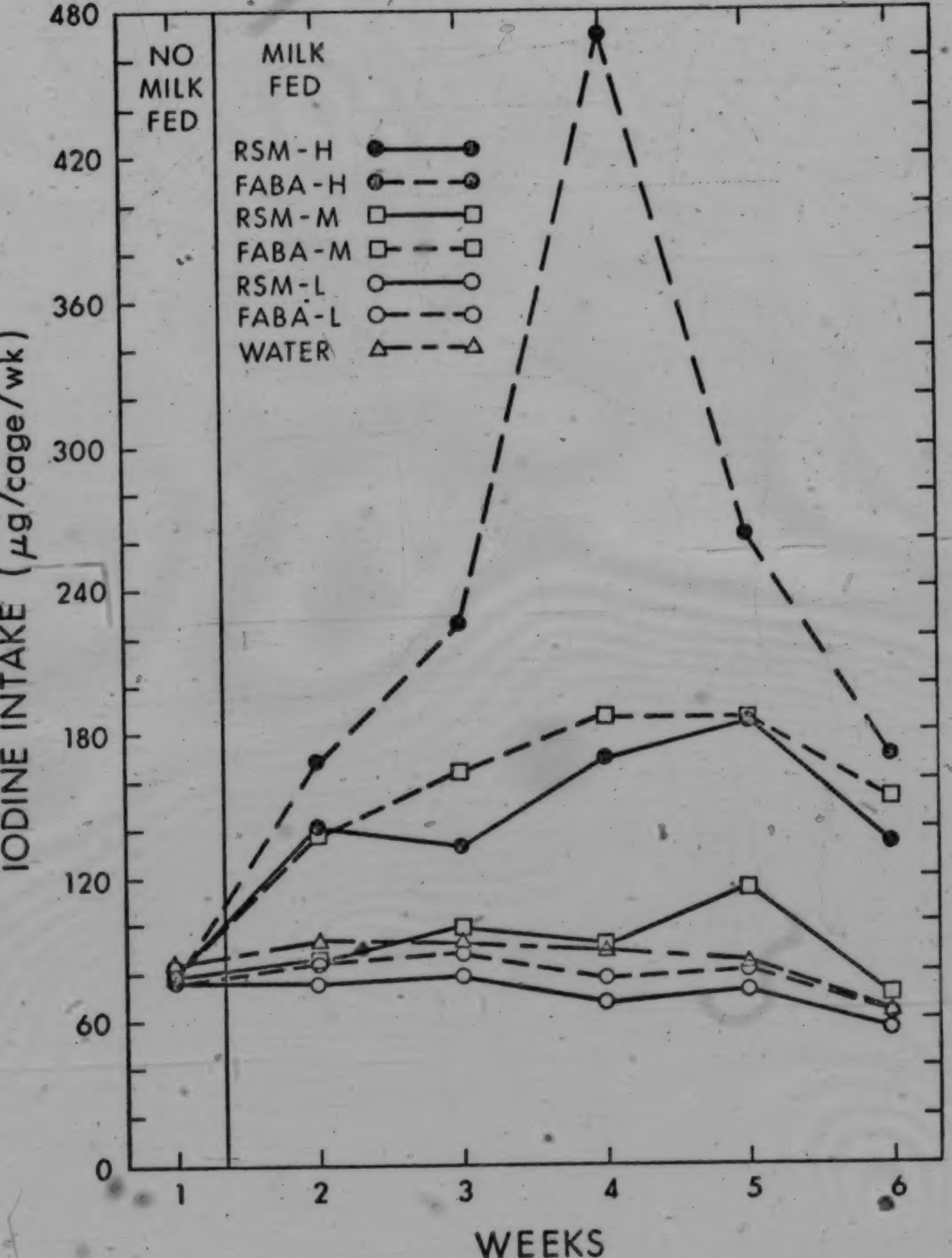

FIGURE 16. Trial III - Weekly Iodine Consumption

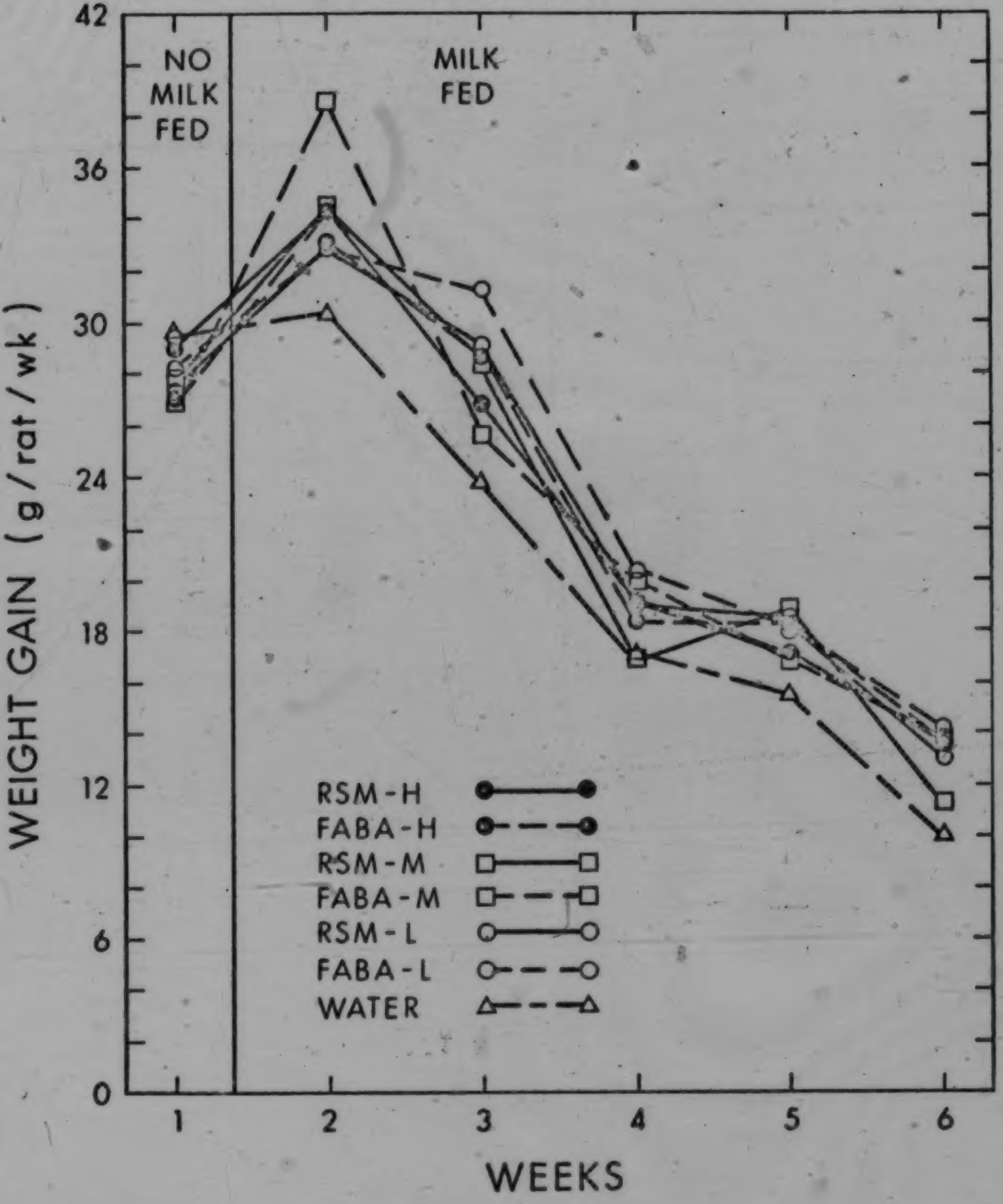

FIGURE 17: Trial III - Weekly Weight Gains

TABLE 18

TRIAL III - GROUP MEANS FOR THYROID GLAND CHARACTERISTICS

Comparison	Group	Thyroid Weight Absolute (mg/rat)	Thyroid Weight Relative (mg/100 g body wt)	Thyroid 48-hr. ^{131}I Uptake Absolute (% of dose)	Thyroid 48-hr. ^{131}I Uptake Relative (% of dose/ 10 mg thyroid wt)
1. Liquids (24 rats per group)	RSM-H	10.5^{ab}	5.0^{bc}	26.41^{b}	25.60^{cd}
	FABA-H	9.7^{bc}	4.6^{cd}	20.62^{b}	21.51^{d}
	RSM-M	10.6^{ab}	5.0^{bc}	41.21^{a}	40.19^{a}
	FABA-M	8.5^{c}	4.0^{d}	25.09^{b}	31.19^{bc}
	RSM-L	11.9^{a}	5.6^{ab}	41.92^{a}	35.47^{ab}
	FABA-L	11.4^{a}	5.2^{abc}	44.21^{a}	38.94^{a}
	WATER	11.7^{a}	5.9^{a}	45.31^{a}	38.30^{ab}
2. Feeds (84 rats per group)	LOW I	11.5^{a}	5.5^{a}	42.23^{a}	36.39^{a}
	HIGH I	9.7^{b}	4.6^{b}	27.70^{b}	29.67^{b}

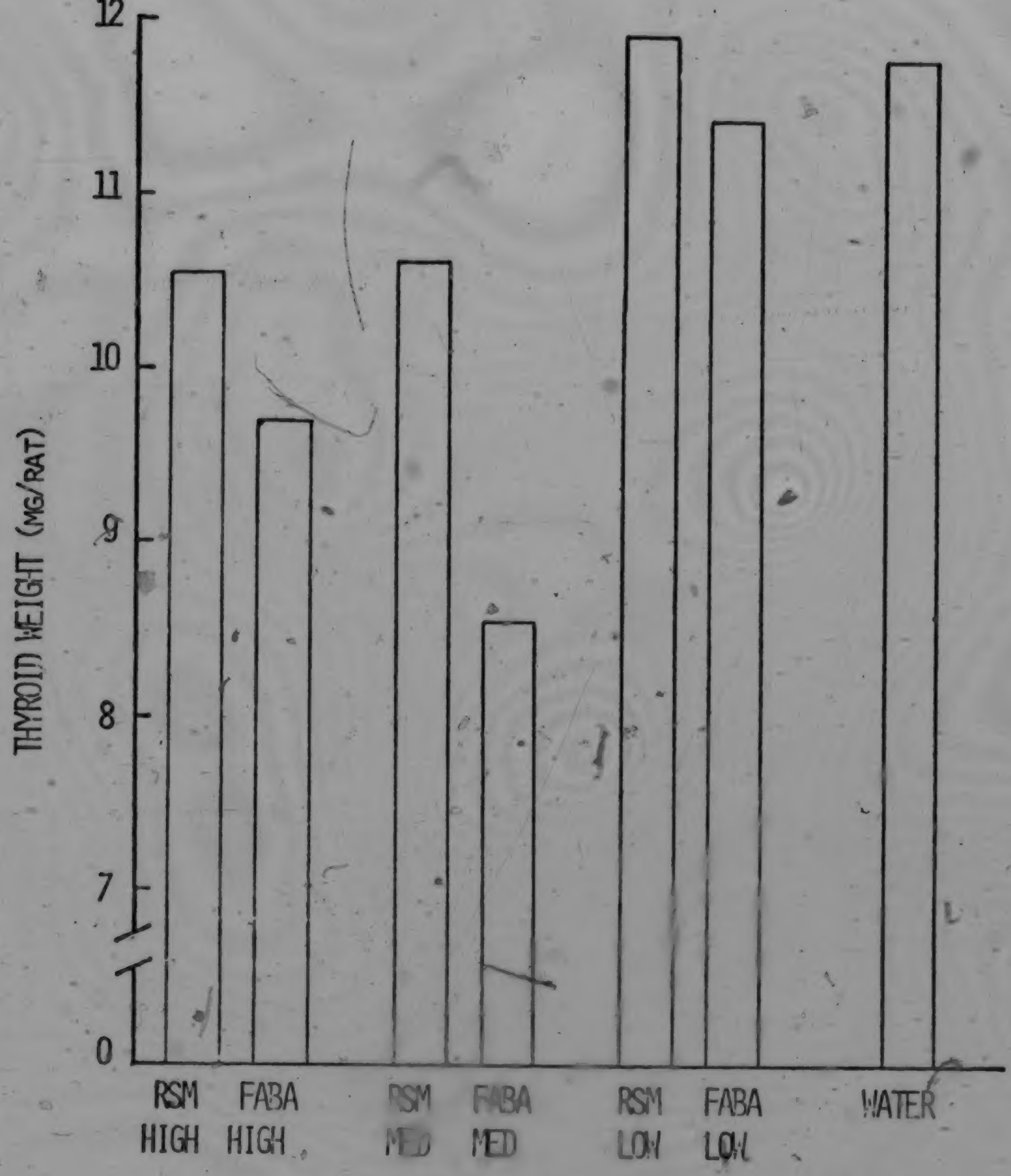

FIGURE 18. TRIAL III - Liquid Group Means for Thyroid Weights

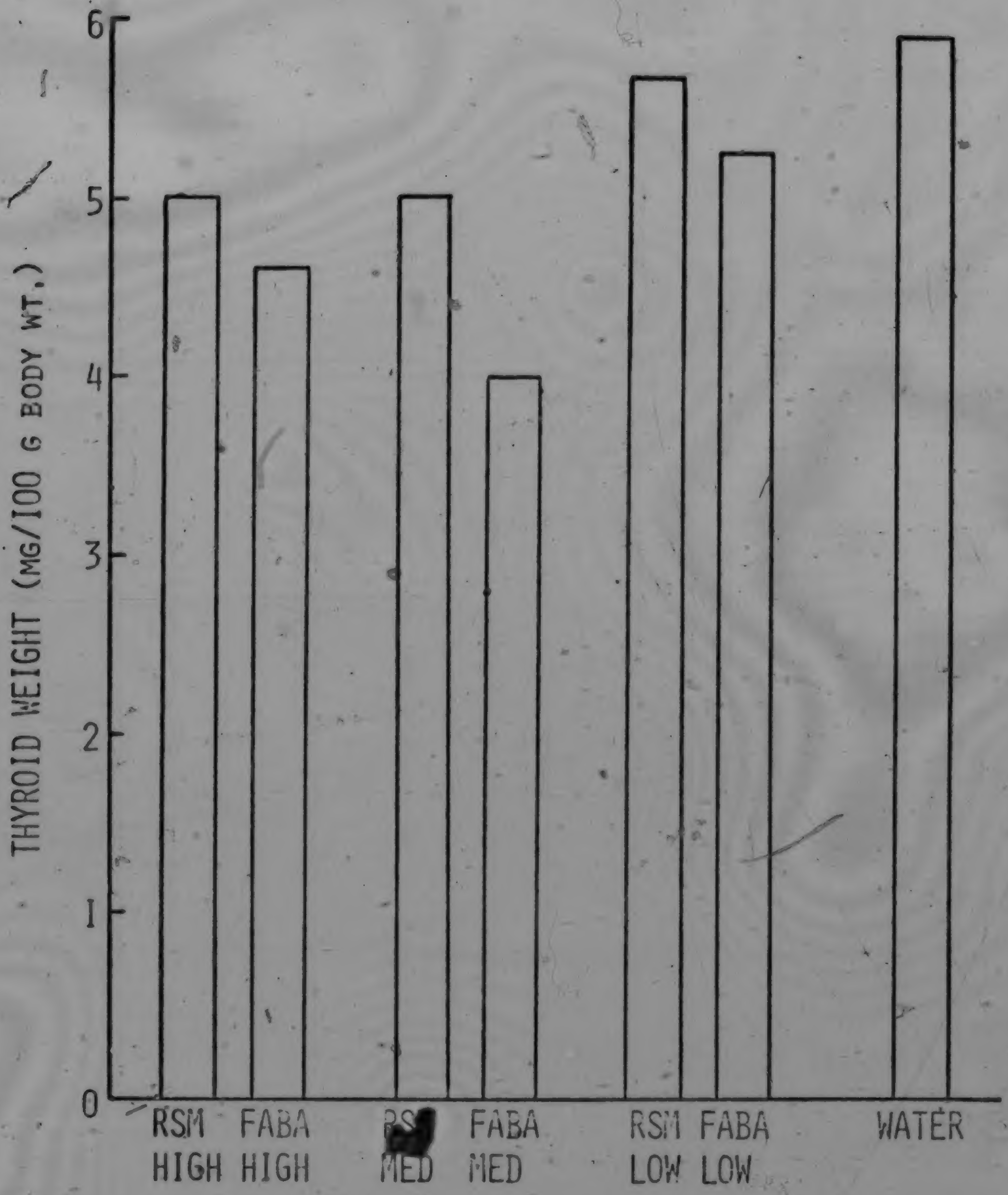

FIGURE 19. TRIAL III - Liquid Group Means for Relative Thyroid Weights

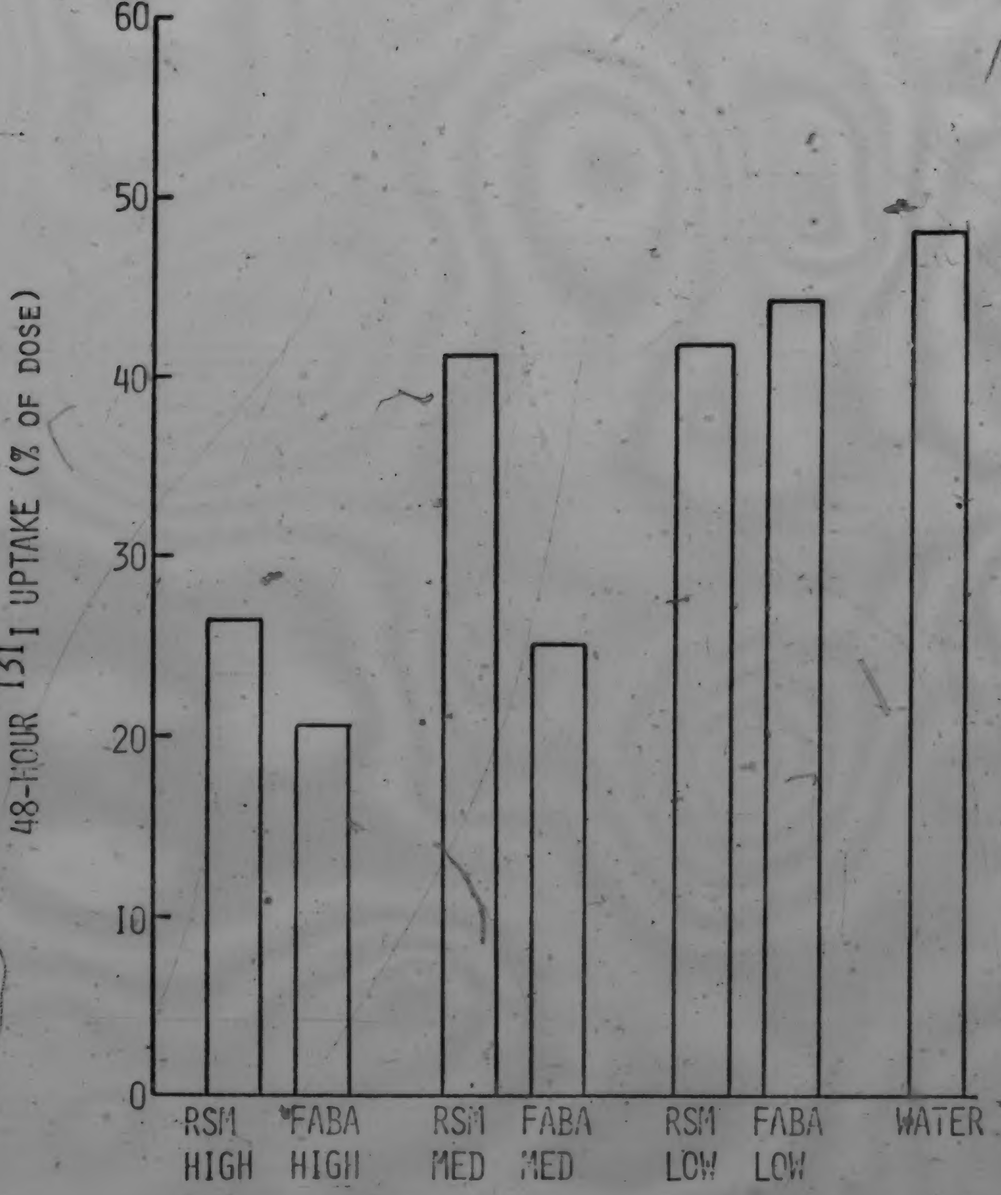

FIGURE 20. TRIAL III - Liquid Group Means for Thyroid Radioiodine Uptake

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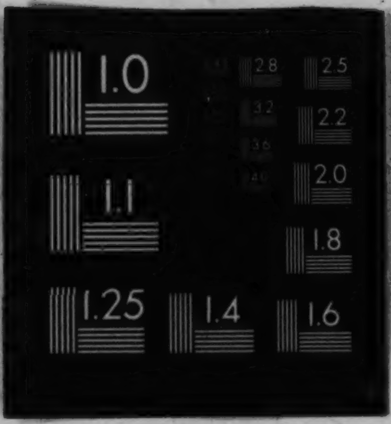

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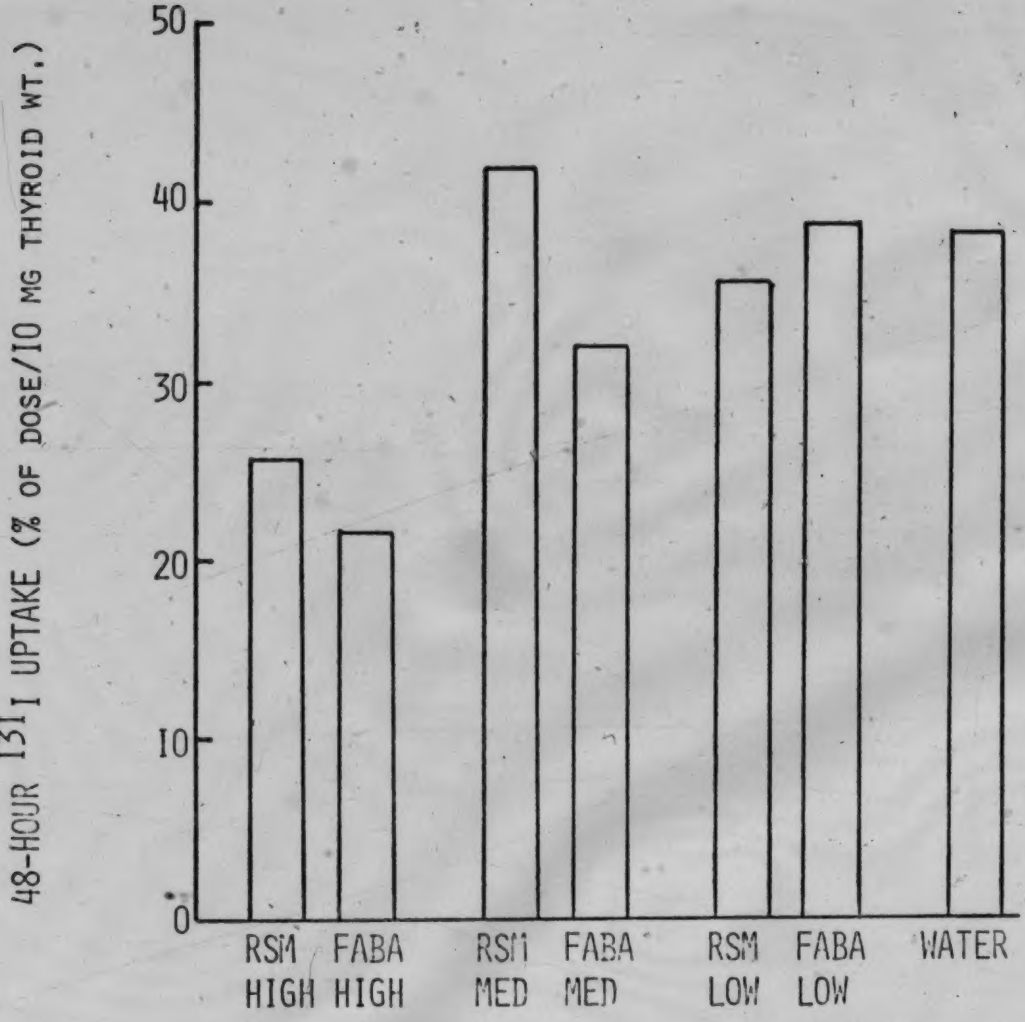

FIGURE 21. TRIAL III - Liquid Group Means for Relative Thyroid Radioiodine Uptake

TABLE 19

TRIAL III - GROUP MEANS FOR SERUM THYROID HORMONE AND RADIOIODINE HALF-LIFE

Comparison	Group	Serum Thyroid Hormone			Thyroid ^{131} I Half-Life Thyroid ^{131}I Half-Life (days)
		T-4 (μg/ 100 ml)	T-3 (ng/100 ml)	T-3/T-4 (ng/100 ml/ μg/100 ml)	
1. Liquids (24 rats per group)	RSM-H	3.8^{a}	89.4^{a}	23.4^{a}	2.21^{ab}
	FABA-H	3.8^{a}	86.2^{a}	23.1^{a}	3.14^{a}
	RSM-M	3.4^{a}	93.7^{a}	28.2^{a}	1.49^{c}
	FABA-M	3.5^{a}	81.2^{a}	26.8^{a}	2.20^{ab}
	RSM-L	3.5^{a}	86.9^{a}	25.1^{a}	1.61^{bc}
	FABA-L	3.4^{a}	81.6^{a}	24.4^{a}	1.56^{c}
	WATER	3.9^{a}	102.9^{a}	27.8^{a}	1.51^{c}
2. Feeds (84 rats per group)	LOW I	3.5^{a}	89.2^{a}	26.2^{a}	1.72^{a}
	HIGH I	3.7^{a}	88.5^{a}	24.9^{a}	1.95^{a}

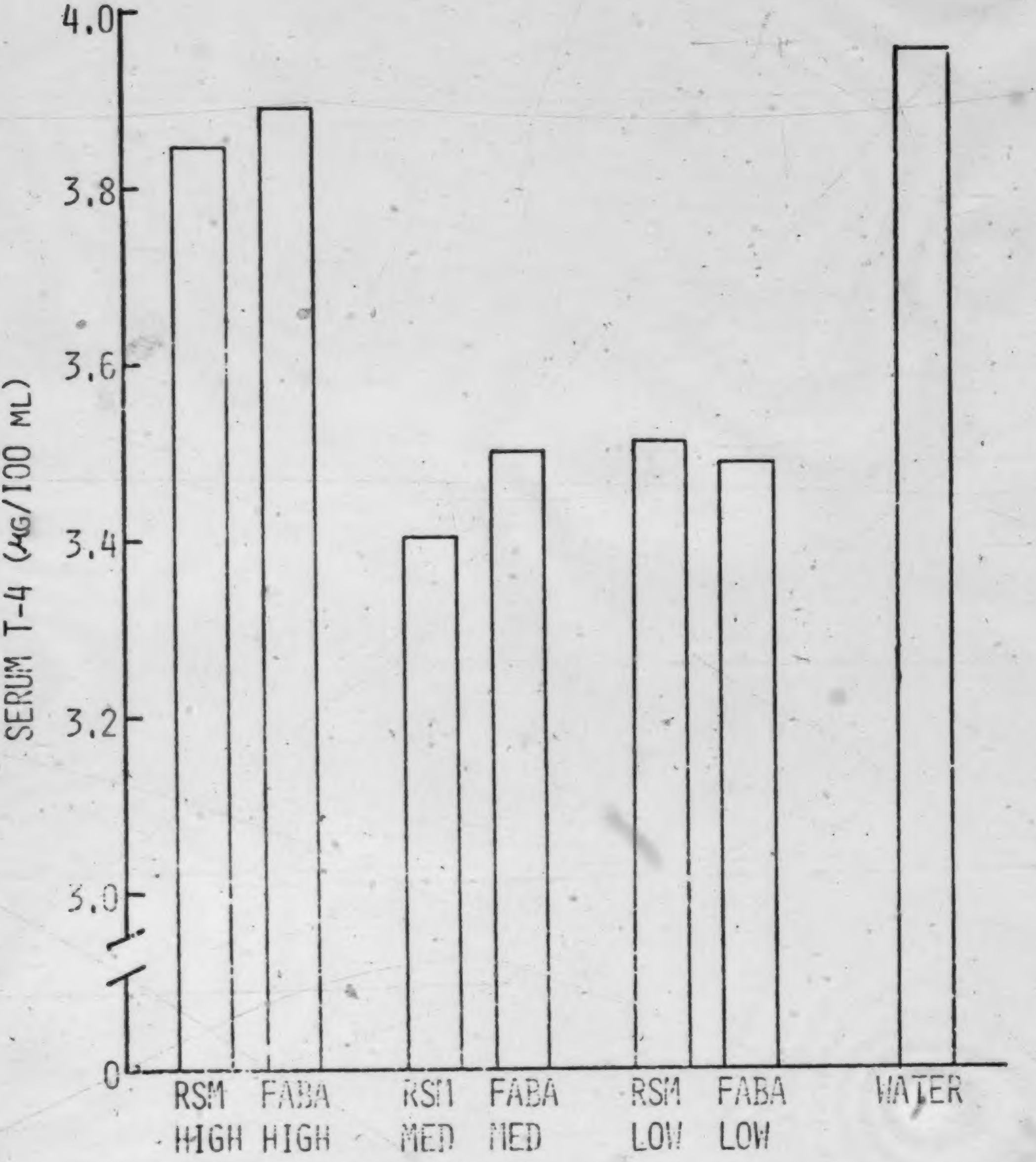

FIGURE 22. TRIAL III - Liquid Group Means for Serum T-4

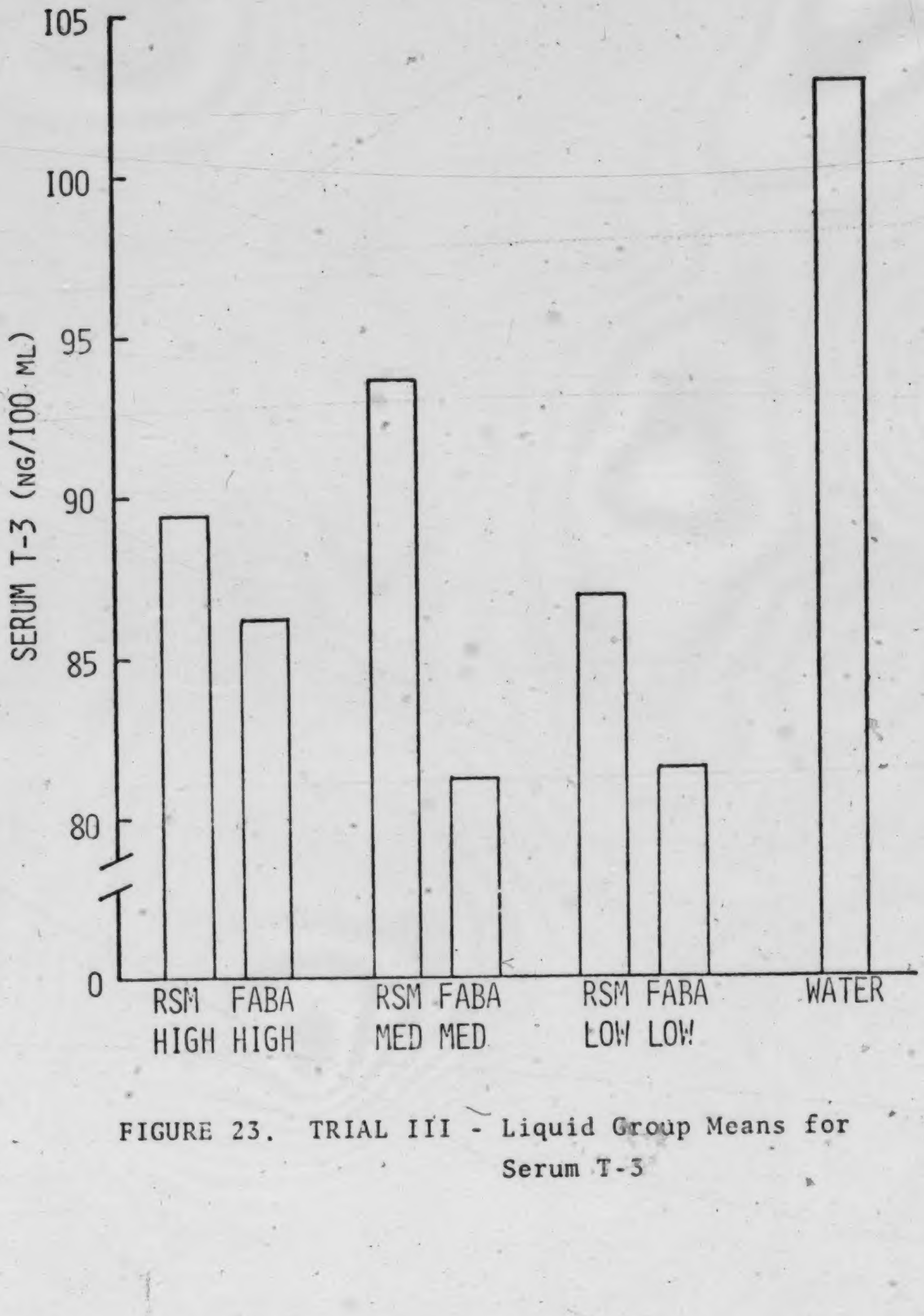

FIGURE 23. TRIAL III - Liquid Group Means for Serum T-3

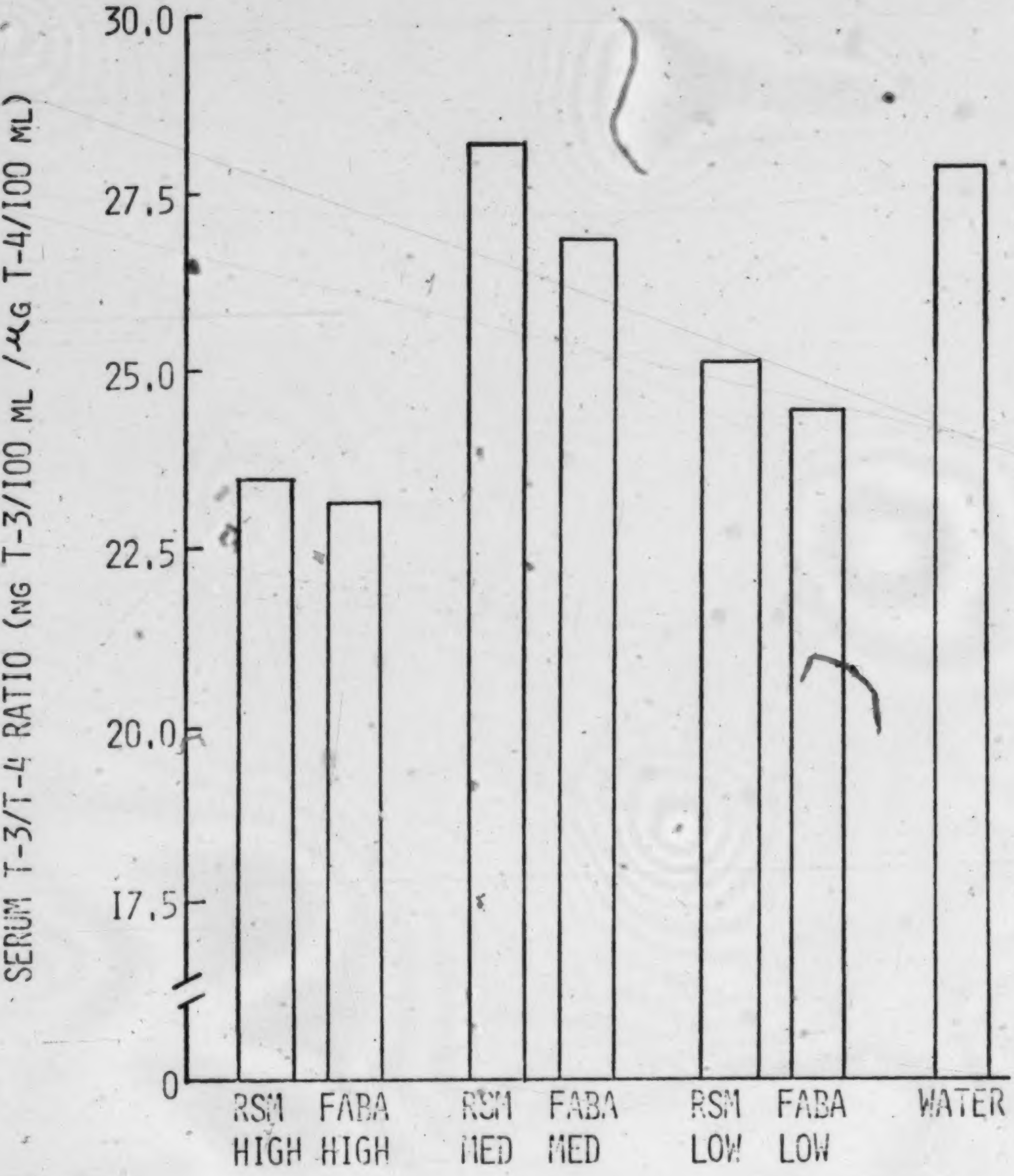

FIGURE 24. TRIAL III - Liquid Group Means for Serum T-3/T-4 Ratio

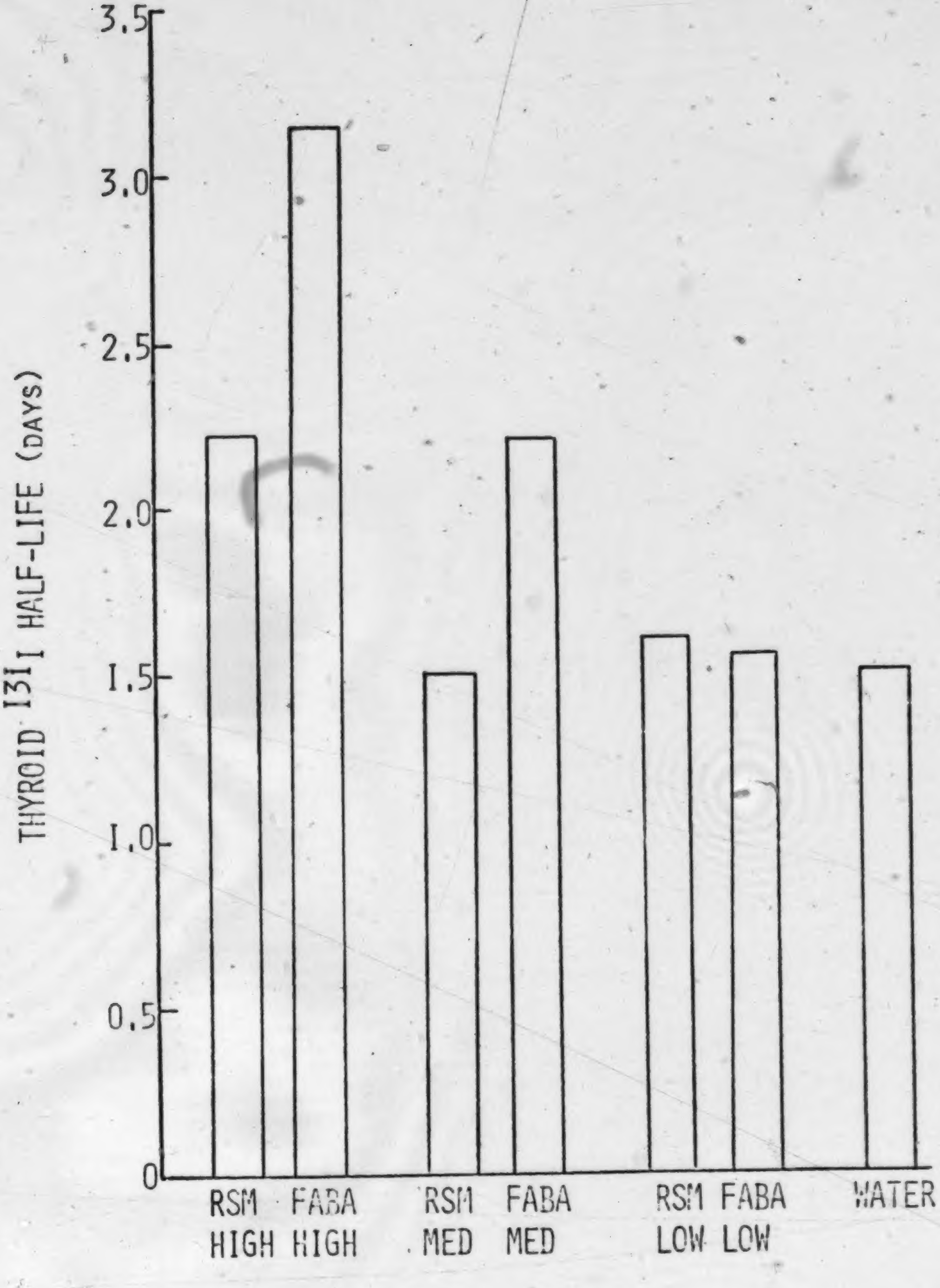

FIGURE 25. TRIAL III - Liquid Group Means for Thyroid Radioiodine Half-Life

TABLE 20

TRIAL III - LIQUID-FEED GROUP MEANS FOR TOTAL FEED, MILK AND IODINE CONSUMPTION, AND TOTAL WEIGHT GAINS

Group Liquid	Group Feed	Feed Intake (g/cage/ week)	Feed Intake (g/rat/ day)	Milk Intake (ml/cage/ week)	Milk Intake (ml/rat/ day)	Iodine Intake (µg/cage/ week)	Iodine Intake (µg/rat/ day)	Weight Gains (g/rat/ week)
RSM-H	LOW I	297	10.6	698	24.9	118	4.2	22.4
FABA-H	LOW I	297	10.6	719	25.7	210	7.5	22.6
RSM-M	LOW I	301	10.8	700	25.0	73	2.6	23.2
FABA-M	LOW I	299	10.7	690	24.6	129	4.6	23.4
RSM-L	LOW I	297	10.6	705	25.2	51	1.8	23.2
FABA-L	LOW I	312	11.1	713	25.5	59	2.1	24.7
WATER	LOW I	391	14.0	0	0	59	2.1	20.8
RSM-H	HIGH I	315	11.3	720	25.7	161	5.8	24.2
FABA-H	HIGH I	302	10.8	715	25.5	249	8.9	24.3
RSM-M	HIGH I	280	10.0	720	25.7	107	3.8	22.7
FABA-M	HIGH I	307	11.0	704	25.1	172	6.1	23.6
RSM-L	HIGH I	299	10.7	702	25.1	90	3.2	23.3
FABA-L	HIGH I	304	10.9	712	25.4	98	3.5	23.5
WATER	HIGH I	386	13.8	0	0	108	3.9	21.0

Each group contains 12 rats.

TABLE 21

TRIAL III - LIQUID-FEED GROUP MEANS FOR THYROID GLAND CHARACTERISTICS

Group		Thyroid Weight		Thyroid 48-hr. ^{131}I Uptake	
Liquid	Feed	Absolute (mg/rat)	Relative (mg/100 g body wt)	Absolute (% of dose)	Relative (% of dose/ 10mg thyroid wt)
RSM-H	LOW I	10.0	4.9	28.16	28.53
FABA-H	LOW I	9.3	4.5	23.39	24.82
RSM-M	LOW I	12.2	5.8	51.26	43.44
FABA-M	LOW I	8.6	4.1	28.48	34.97
RSM-L	LOW I	13.8	6.6	52.80	39.25
FABA-L	LOW I	12.4	5.6	52.76	43.36
WATER	LOW I	14.3	7.2	58.78	40.40
RSM-H	HIGH I	11.0	5.1	24.65	22.68
FABA-H	HIGH I	10.0	4.6	17.84	18.20
RSM-M	HIGH I	8.9	4.3	31.15	36.95
FABA-M	HIGH I	8.4	3.9	21.70	27.42
RSM-L	HIGH I	9.9	4.7	31.05	31.68
FABA-L	HIGH I	10.4	4.8	35.65	34.53
WATER	HIGH I	9.1	4.6	31.84	36.20

Each group contains 12 rats.

TABLE 22

TRIAL III - LIQUID-FEED GROUP MEANS FOR SERUM THYROID HORMONE AND RADIOIODINE HALF-LIFE

Group		Serum Thyroid Hormone			Thyroid ^{131}I Half-Life
Liquid	Feed	T-4 (µg/ 100 ml)	T-3 (ng/ 100 ml)	T-3/T-4 (ng/100 ml/ µg/100 ml)	Thyroid ^{131}I Half-Life (days)
RSM-H	LOW I	3.8	81.1	20.7	2.30
FABA-H	LOW I	3.9	83.4	21.6	2.62
RSM-M	LOW I	3.1	95.8	31.0	1.43
FABA-M	LOW I	3.6	81.1	24.4	2.13
RSM-L	LOW I	3.4	94.7	28.1	1.46
FABA-L	LOW I	3.0	82.4	28.4	1.49
WATER	LOW I	3.8	106.3	29.3	1.39
RSM-H	HIGH I	3.8	97.7	26.2	2.13
FABA-H	HIGH I	3.8	89.1	24.7	3.92
RSM-M	HIGH I	3.6	91.6	25.4	1.54
FABA-M	HIGH I	3.3	81.4	29.2	2.27
RSM-L	HIGH I	3.5	79.0	22.0	1.79
FABA-L	HIGH I	3.9	80.9	20.4	1.63
WATER	HIGH I	4.0	99.5	26.4	1.66

Each group contains 12 rats.

group which, because of its higher feed intake, actually consumed more iodine than the RSM-L group (Figure 16). All differences between liquid groups were significant except the one between the FABA and WATER groups (Table 17). In the feed groups comparison, the Low I group consumed significantly less iodine than the High I group.

7. Weight gains. There were no significant differences in weight gains between any of the liquid groups (Table 17). There was also no significant difference in weight gain between the Low I and High I feed groups.

8. Thyroid weights (absolute). Table 16 shows that overall, the RSM-milk resulted in significantly larger thyroids than the FABA-milk. Breaking this difference down into the six individual types of milk reveals that all RSM groups had higher thyroid weights than their respective FABA groups, but the only significant difference was between the RSM-M and FABA-M (Table 18). This difference appeared mainly in the Low I feed group since the RSM-M-High I group had a mean thyroid weight not appreciably higher than FABA-M-High I group. The Low I feed group had significantly higher thyroid weights than the High I feed group.

9. Thyroid weights (relative to body weight). Most of the differences noted for the absolute thyroid weights were found in the relative thyroid weights. The RSM group had significantly greater relative thyroid weights overall than the FABA group (Table 16). Figure 19 shows that the

individual RSM groups had higher relative thyroid weights than their respective FABA groups, but that this difference was only significant in the comparison of the RSM-M and FABA-M (Table 18). As in the absolute thyroid weights, the difference between the RSM-M and FABA-M was accounted for in the Low I feed groups (Table 21). Table 18 shows that no significant difference was found in the relative thyroid weights in the feed groups comparison.

10. 48-hour radioiodine uptake (absolute). In the overall liquids comparison, the RSM group had a significantly greater absolute 48-hour radioiodine uptake than the FABA group (Table 16). Figure 20 and Table 18 reveal that most of this difference was due to the milks produced by cows fed on the H and M salt mixes, and the M group was the only one where the RSM-milk caused a significantly greater radioiodine uptake than the FABA-milk. Generally, except for the RSM-L group, the seven liquid groups followed the inverse order of their total iodine intake. In the feeds comparison, the Low I group had a significantly higher radioiodine uptake than the High I group (Table 18).

11. 48-hour radioiodine uptake (relative to thyroid weight). When the radioiodine uptake was put on a thyroid weight basis, the overall RSM group was not significantly higher than the FABA group (Table 16). The RSM-M group had a significantly higher relative radioiodine uptake than the FABA-M group (Table 18). In the feeds comparison, the Low I group had a significantly higher mean relative radioiodine

uptake than the High I group (Table 18).

12. Serum T-4 concentrations. There were no significant differences in serum T-4 concentrations for the overall comparison of RSM and FABA (Table 16), or in the individual liquids comparisons (Figure 22 and Table 19). The Low I feed caused a lower mean serum T-4 concentration, but the difference was not significant (Table 19).

13. Serum T-3 concentrations. The RSM groups all had higher serum T-3 concentrations than their respective FABA groups (Figure 23), but the differences between the individual groups (Table 19), and in the overall comparison of RSM and FABA were not significant. The feeds comparison yielded no significant differences in serum T-3 concentrations (Table 19).

14. Serum T-3/T-4 ratios. As in the T-3 comparison, the RSM liquid groups had higher serum T-3/T-4 ratios than their respective FABA groups (Figure 24), but there were no significant differences between any of the liquids (Table 19) or in the overall comparison (Table 16). In the feeds comparison, the Low I group had a higher serum T-3/T-4 ratio than the High I group, but the difference wasn't significant (Table 19).

15. Thyroid radioiodine half-life. The RSM group had a significantly shorter overall thyroid radioiodine half-life than the FABA group (Table 16). When the individual liquids were examined, most of the differences occurred in

the H and M salt mixes (Figure 25). The RSM-M caused a significantly shorter half-life than the FABA-M, but this was the only significant difference within an individual salt mix grouping (Table 19). The Low I feed group had a shorter mean radioiodine half-life than the High I group, but this difference wasn't significant (Table 19).

DISCUSSION

MILK IODINE CONCENTRATION

The results of these trials clearly show that RSM-fed cows produced milk which was lower in iodine concentration than the milk produced by cows consuming faba beans. This effect was consistent over a wide range of feed iodine supplementation ranging from no supplemental iodine (Trial III), to NRC (1971) supplemental iodine requirements (Trials II and III), to 15-times NRC (1971) supplemental iodine requirements (Trial I). The feeding of Bronowski rapeseed meal in Trial II also led to depressed milk iodine concentrations.

Results from the literature reveal possible causes for this RSM effect. Piironen and Virtanen (1963) found that cows consuming thiocyanates produced milk with a depressed iodine concentration. Hemken et al. (1972) suspected thiocyanates as being responsible for the soybean meal-induced reduction of milk iodine levels. Iwarsson (1974) found that low iodine milk from certain areas of Sweden contained high levels of thiocyanates. Since thiocyanates are also found in rapeseed and rapeseed meal (Iwarsson, 1973), they are the probable cause of the lowered milk iodine levels found in these trials.

In both Trials II and III, the RSM-fed cows produced more milk than the FABA-fed cows. Garner et al. (1960) found that the iodine concentration of a cow's milk is inversely proportional to her production. This finding could explain at least part of the depression in the iodine concentration of the RSM-milks. However, the comparisons of the BRON-milk and FABA-milk in Trial II, and the RSM-H-milk and the FABA-H-milk in Trial III, reveals that in both cases the lower producing cows produced milk with the lowest iodine concentration. These comparisons indicate that most of the differences in iodine concentration between the RSM- and FABA-milks were not due to production differences.

The second important trend from the milk iodine analysis data is that the iodine content of the milk produced from a particular kind of protein supplement varied directly with dietary iodine consumption. This effect can be seen most clearly in the comparison of the milks produced on the various salt mixes in Trial III. The milk iodine concentrations of RSM-L (0.9 μg I/100 ml) and FABA-L (1.7 μg I/ 100 ml are (relative to the other milks in Trial III) close to the value of 0.8 μg I/100 ml found when cows were fed an iodine-deficient diet by Hemken et al. (1971) and Miller and Swanson (1973). By the standards of Alderman and Stranks (1967), cows producing milk with an iodine concentration below 2.5 μg/100 ml are considered to be iodine deficient. Since the RSM-L and FABA-L groups of Trial III were producing milk below this standard, it could be assumed that they would become hypothyroid if left on these rations for long periods of time.

The STORE-milk from Trial II averaged 23.6 μgI/100 ml, revealing that the average cow in the Edmonton milkshed is receiving sufficient iodine. According to the work of Miller and Swanson (1973), this iodine concentration corresponds to a feed level of approximately 65 mg I/cow/day which would be almost 5-times NRC (1971) requirements for a cow consuming 20 kg of dry matter daily. The results of Trial I show that the high iodine level of this milk could be due to the feeding of high iodine salt for foot-rot prevention. The use of the popular iodophor teat dips could also be partially responsible (Iwarsson and Ekman, 1974).

RAT FEED AND MILK CONSUMPTION AND WEIGHT GAINS

The WATER groups, because they were obtaining no energy in their liquid ration, were expected to consume more solid feed than the milk groups. This effect showed up quite clearly in Trials II and III, but there were no differences in feed consumption between any of the milk groups in an individual trial.

The only difference in milk consumption occurred in Trial II, where the FABA group drank significantly less milk than the other groups. No explanation for this finding can be offered at this time, but Table 11 and Appendix A-1 point out that both males and females in the FABA-Low I group were responsible for this difference.

No differences were found in weight gains between the milk groups, but the milk groups all gained faster than the WATER groups in Trials II and III. This observation points out that the nutritional value of the milk to promote growth was better than the extra solid feed consumed by the WATER groups.

Iwarsson and Nilsson (1973) also found no differences in feed or milk consumption or total weight gain between rats fed RSM-milk and rats fed soybean meal (SBM)-milk. These authors did find however that the RSM-milk promoted higher weight gains during the first week on trial. In the study reported herein, this effect was noted in Trial II but not in Trials I or III.

The general conclusion that can be drawn from these findings is that if RSM-milk has any goitrogenic properties, they are not adversely affecting the rats at the feed consumption or weight gains level as occurs when rapeseed meal is fed to poultry or swine (Clandinin, Robblee, and Slinger, 1972; Bowland and Bell, 1972).

In the secondary comparisons, the males consumed more feed in Trials I and II, more milk in Trial I, and grew faster in Trials I and II. These results were expected since in most species, the males consume more feed and grow faster than the females.

In the feed groups comparison, there was no significant difference in feed consumption or weight gains in Trials II or III and no difference in milk consumption in Trial III. The fact that the High I group in Trial III drank more milk is difficult to explain, especially since the moisture and gross energy of the feeds were examined and found to be the same.

IODINE INTAKE

The NRC (Warner and Breuer Jr., 1972) lists the minimum iodine requirement to stop thyroid enlargement in the laboratory rat as being 0.15 mg I/kg feed. Of the feeds used in these trials, only the wheat-starch ration used throughout Trial I and as the Low I diet in Trial II was significantly below this (0.04 mg I/kg). The Low I feed of Trial III (0.15 mg I/kg) met requirements exactly, the High I feed of Trial III (0.28 mg I/kg) contained twice the requirements, and the High I feed of Trial II (2.0 mg I/kg) contained about 13-times requirements.

The average feed consumption of all rats consuming water in these trials was 16.0 g/rat/day. The iodine consumption of these rats if NRC minimum iodine requirements were met would be 2.4 μg I/rat/day. This agrees well with the published estimate of 1-2 μg/rat/day (Levine et al., 1933). When the total iodine consumption from milk and from feed is calculated for the rats in these trials, only the

WATER-Low I (0.6 μg/rat/day) in Trial II, and the RSM-L-Low I (1.8 μg/rat/day), FABA-L-Low I (2.1 μg/rat/day), and the WATER-Low I (2.1 μg/rat/day) in Trial III are below 2.4 μg/rat/day. If the estimates of requirements are accurate, only these groups were susceptible to thyroid inhibition caused by iodine deficiency. Thyroid problems occurring in other groups indicate other problems such as dietary goitrogens.

SINGLE MEASUREMENT PARAMETERS OF THYROID INHIBITION

The single measurement parameters provide data for the comparison of time periods, sexes, feed iodine levels, and liquids. Since the first two are secondary to the main object of this study, they shall be discussed separately from the liquids comparison. The feed iodine levels shall only be discussed as they affect the liquids comparison.

Secondary Comparisons

The time period comparison of Trial I was done to discover which trial length, 2 weeks or 5 weeks, was best to evaluate thyroid function. No significant differences were found for thyroid weights or radioiodine uptakes, but there was a significant and unexplainable depression in the PBI values for the 2 week rats. This result was not expected since the thyroid storage of thyroid hormones is normally enough to buffer PBI concentrations for several weeks (Tepperman, 1973). No conclusions were drawn from this data, but it was decided to run all trials with a 5 week milk feeding period to enable a comparison with the results of Iwarsson and Nilsson (1973). The possibility that a 2 week trial would be sufficient for determining differences in thyroid function deserves further investigation.

The results of the sex comparisons imply that females reveal thyroid inhibition more readily than males. The females displayed higher thyroid weights in both Trials I and II, lower PBI concentrations in Trial I, and lower serum T-4 values and elevated serum T-3/T-4 ratios in Trial II. Human females are thought to be more susceptible to thyroid inhibition than males (Koutras, 1971). Much of this difference was thought to be due to the increased iodine losses resulting from pregnancy, lactation, and menstruation (Wayne et al., 1964). Since the rats in these trials were neither pregnant, lactating, nor menstruating, the results support those of Grosvenor (1962) who found that more direct hormonal aspects are responsible for the difference in susceptibility to thyroid inhibition of male and female rats.

Liquids Comparison

The results of Trials II and III indicate that RSM-milk caused larger absolute and relative thyroid weights than the FABA-milk. Iwarsson and Nilsson (1973) obtained similar findings when milk from cows consuming Swedish RSM was compared to milk from cows consuming soybean meal (SBM), and they implicated the relative iodine deficiency of the

RSM-milk as being responsible.

In the study reported herein, differences in the milk iodine concentrations may account for much of the difference in thyroid size. High levels of iodine in the milk (Trial I) or in the rat feed (Trial III) removed much of the difference in thyroid weights between the RSM and FABA groups.

These trials show that the NRC iodine requirement for rats (Warner and Breuer Jr., 1972) is an accurate estimate of the level which will just stop significant thyroid enlargement. All four groups in Trials II and III which were calculated to be consuming inadequate amounts of iodine had significantly enlarged thyroids, whereas all but two of the groups (RSM groups) consuming sufficient iodine showed no significant thyroid enlargement. Iwarsson and Nilsson (1973) found that all of their rats had iodine requirements above those recommended by NRC (Warner and Breuer Jr., 1972). This could be because both their control protein source (SBM) and their test protein source (RSM) are from plant sources known to contain goitrogens (Van Etten, 1969).

The fact that the RSM-Low I group of Trial II and the RSM-M-Low I group of Trial III were the only groups which showed appreciable thyroid enlargement when iodine intake was above requirements reveals that there were likely goitrogens in the RSM-milk. Other comparisons indicate that in Trial II, goitrogens may have played a larger role than iodine deficiency in promoting enlarged thyroids in the RSM groups. The RSM-milk caused larger thyroids than the BRON-milk, even though the latter had a slightly lower iodine concentration. The RSM-milk also caused larger thyroids than the FABA controls in both the Low I and High I groups, even though the High I feed contained 13-times the rat requirement for iodine.

The exact nature of the goitrogen present is difficult to determine from the thyroid weight data. Peltola (1960) and Krusius and Peltola (1966) found that iodine supplementation up to 150 μg I/rat/day could not eliminate thyroid enlargement due to goitrin. The ineffectiveness of the High I diet to overcome the thyroid enlargement due to RSM-milk in Trial II also implicates a thionamide goitrogen such as goitrin. Thiocyanate involvement is a possibility, especially in Trial III since the goitrogen effects weren't as evident when the High I feed was consumed. Iwarsson and Nilsson (1973) concluded that thiocyanates weren't responsible for the thyroid enlargement which occurred in the RSM groups of their trials.

The radioiodine uptake data reveals differences in the goitrogenic potential of the RSM- and FABA- milks, but the nature of the causes is not clear. In Trial I, the significant difference in relative radioiodine uptake was due to the lower iodine level of the RSM-milk. In Trial III, the significant difference in the absolute radioiodine uptake was due to the iodine-deficiency of the RSM-milks. This conclusion is reinforced by the finding that substituting the High I feed for the Low I feed removed the significant difference between the RSM-M and the FABA-M in Trial III. These results agree with those of Iwarsson and Nilsson (1973) who also found increased radioiodine uptakes when rats were fed RSM-milk.

The results from Trial II indicate that goitrogenic factors were present in the RSM-milk. The significantly lower relative radioiodine uptake indicates that each unit of the thyroid mass was trapping less iodine than in the FABA or BRON groups. The difference between the RSM and BRON groups can be explained by iodine differences since the uptakes weren't different when the High I feed was consumed (Table 12). The RSM-milk however caused a lower uptake than the FABA-milk in both the Low I and High I feed groups. Since the RSM-milk contained less iodine than the FABA-milk, it therefore must have contained some goitrogen which blocked either the trapping or formation of organic iodine.

The evidence from the thyroid weights and the thyroid radioiodine uptakes of Trial III are in conflict on the question of goitrogens in the RSM-milk. Krusius and Peltola (1966) concluded that radioiodine uptake is a much less sensitive parameter for detecting small levels of ingested goitrin than is thyroid weight measurement. Thus it is possible that the low levels of goitrogen in the RSM-milks which caused the differences in thyroid weights, were not high enough to overcome the elevated radioiodine uptake effect of their relative iodine deficiency.

The RSM-milk of Trial II must have contained a high level of a goitrogen, and the failure of iodine supplementation to eliminate effects implicates goitrin. Krusius and Peltola (1966) found that daily goitrin administrations of up to 2 μg/rat would not cause depressed thyroid radioiodine uptakes and so it must be assumed that the RSM group in Trial II was ingesting more than this amount. Since the milk intake of Trial II averaged 33.4 ml/rat/day, the minimum milk goitrin concentration to cause a daily ingestion greater than 2 μg/rat/day would be 6 μg/100 ml. Virtanen et al. (1958) found goitrin concentrations up to 10 μg/100 ml when a cow consumed 4.5 g of goitrin in 500 g of raw rapeseed. If the RSM in Trial II contained about 4.0 mg goitrin/g of feed (Iwarsson, 1973; Lo and Bell, 1972), a cow eating 1660 g of RSM from 10 kg of grain would ingest about 6.5 g/day of goitrin. This theoretical calculation shows that goitrin levels well in excess of 6 μg/100 ml were possible for the RSM-milk in these trials. It therefore is possible for the rats in the RSM group to have consumed much more goitrin per day than the upper level tested by Krusius and Peltola (1966).

The fact that thiocyanates in interaction with goitrin could be involved in Trial II cannot be overlooked. There are however no values in the literature on the doses of thiocyanates which must be chronically administered to depress thyroid radioiodine uptake and so no statement can be made regarding their presence.

The results of the serum thyroid hormone analysis reveal that generally RSM-milk has a goitrogenic effect, but over the time period of these trials, the animals were able to adjust to maintain euthyroidism. The RSM-milk caused depressed PBI concentrations (Trial I) and serum T-4 concentrations (Trials II and III), and elevated serum T-3 concentrations and serum T-3/T-4 ratios (Trials II and III), but none of these effects were significant. Iwarsson and Nilsson (1973) obtained similar results since the PBI concentrations in their RSM groups were depressed significantly in only one trial out of three. Whether the results were caused by a goitrogen in the RSM-milk or just a relative iodine deficiency cannot be ascertained since both affect serum thyroid hormone concentrations similarly.

The results from Trial III show that there was a significant RSM-milk effect on shortening the radioiodine half-life. As with the other results of Trial III, most of these differences occurred in the M salt mix comparison which was the only one in which borderline and adequate iodine intakes were compared. For this reason, and because the short radioiodine half-lives occurred in the same groups which had high 48-hour radioiodine uptakes, it is concluded that the differences in iodine between the RSM- and FABA-milks were causing the differences in thyroid radioiodine half-lives between these groups.

An overall comparison seems to indicate that the RSM-milk used in this study had more goitrogenic potential (particularly in Trial II) than that of Iwarsson and Nilsson (1973). Although this may reveal a difference between Canadian and Swedish RSM, it should be remembered that RSM made up 16.6% of the concentrate ration in this study, while the highest percent RSM in the Swedish study was 8.05%. This may explain why Iwarsson and Nilsson (1973) found very little evidence of goitrogens in the RSM-milk as compared to the present study.

GENERAL CONCLUSIONS

The results of this study show that milk from cows consuming Canadian rapeseed meal had goitrogenic properties when fed to rats. These properties resulted in significantly increased thyroid weights, abnormal radioiodine uptakes, and shortened thyroid radioiodine half-lives. Although slight depressions in serum T-4 and elevations in serum T-3 also occurred, over a five week period the animals were generally able to maintain euthyroidism and showed normal feed consumption and growth rates.

The most important single cause of rat thyroid inhibition in these studies was the lowered iodine content of the RSM-milk. Two of the three trials showed that the goitrogenic properties could be largely overcome by increasing the iodine content of the feed or milk. Since the iodine content of a cow's milk was shown to vary directly with the iodine content of her diet, this latter result can be accomplished by supplementing the cow's diet with iodine.

One trial out of the three reported herein (Trial II) revealed thyroid inhibition beyond that caused by simple iodine deficiency. The goitrogenic compounds known to be found in rapeseed meal must be suspected of causing these additional problems. Of these, goitrin is the most likely to be involved since attempted iodine prophylaxis was not entirely effective at overcoming the RSM-milk effect on thyroid enlargement or radioiodine uptake.

There are several implications of these findings. As regards dairy cow feeding, rapeseed meal appears to increase the cow's iodine requirments as other members of the Brassica family do (Piironen and Virtanen, 1963). Although the present NRC (1971) recommendations for iodine supplementation are adequate, it must be remembered when formulating rapeseed meal rations, that these standards are minimum and must be met. The use of noniodized salt over a five week trial caused milk iodine levels below the standard set for measuring iodine deficiency in cattle (Alderman and Stranks, 1967) and meeting only half of the NRC requirements resulted in milk iodine levels not appreciably above this standard. Since iodine up to 1000 mg/day has been shown to cause no toxic effects in cows (Miller and Swanson, 1973), meeting the ARC (British Agricultural Research Council, 1965) requirements of 2.0 mg I/kg DM may be a safer practice than using the NRC (1971) recommendations.

The results of this trial also have implications on human nutrition. If the lowered iodine level in RSM-milk is the only problem, this can be overcome by feeding more iodine either to the cows or to the humans consuming the milk. Since the role of milk as a source of iodine has decreased in importance, even if the low iodine content of the milk was not corrected at the farm level, it is unlikely that there would be a widespread outbreak of goitre. But for young children that may receive most of their iodine requirements from milk, some other method of supplementing the diet may have to be found.

If goitrogens, especially goitrin are involved, the situation becomes much more serious. Some evidence (Peltola, 1960) indicates that the consumption of chronic low levels of goitrin (such as would be consumed via milk) may cause thyroid inhibition which cannot be entirely overcome by iodine supplementation. Although most people would be able to adjust to this situation by mild thyroid enlargement, Tepperman (1974) warns that whenever thyroid hypertrophy occurs the chances of eventual thyroid carcinoma are increased.

The fact that only one out of the three trials revealed serious goitrogen problems, indicates that the rapeseed meal consumed by the cows in this study may have been variable with respect to goitrogen content. Since it is known that improper processing of rapeseed can result in a meal high in goitrogens, it is possible that proper care in processing can result in a meal which will not cause goitrogens to get into the milk. The feeding of dairy cows with untreated rapeseed, such as would be available when harvesting conditions are poor, should be investigated with respect to the effects it has upon the goitrin content of milk before any widespread recommendations are made.

As a final conclusion, the limitations of this study should be kept in mind when interpretations are being made. The effect of Canadian rapeseed meal upon the iodine content of a cow's milk, and the subsequent effects of this milk when fed to an animal receiving an otherwise iodine-deficient diet, were established beyond reasonable doubt. The conclusions drawn regarding the goitrogens in this milk were indirect however and more research is needed to establish actual goitrogen levels in milk from cows consuming Canadian rapeseed meal.

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APPENDICES

APPENDIX A

TRIAL II CAGE DATA

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APPENDIX A-1

TRIAL II - CAGE MEANS FOR TOTAL FEED, MILK AND IODINE CONSUMPTION AND TOTAL WEIGHT GAINS

Liquid	Cage Feed	Sex	Feed Intake (g/cage/ week)	Milk Intake (ml/cage/ week)	Iodine Intake (µg/cage/ week)	Weight Gains (g/rat/ week)
RSM	LOW I	F	325	930	83	21.6
RSM	LOW I	M	529	938	92	37.9
RSM	HIGH I	F	356	938	785	17.7
RSM	HIGH I	M	522	938	1106	37.9
FABA	LOW I	F	389	851	125	23.0
FABA	LOW I	M	537	736	115	37.9
FABA	HIGH I	F	304	937	709	12.7
FABA	HIGH I	M	515	938	1131	36.2
BRON	LOW I	F	351	905	75	23.9
BRON	LOW I	M	551	922	73	38.8
BRON	HIGH I	F	330	938	708	12.0
BRON	HIGH I	M	534	938	1115	32.5

APPENDIX A-1 (Continued)

Liquid	Cage Feed	Sex	Feed Intake g/cage/ week)	Milk Intake (ml/cage/ week)	Iodine Intake (µg/cage/ week	Weight Gains (g/rat/ week)
WATER	LOW I	F	485	0	19	13.4
WATER	LOW I	M	408	0	16	8.6
WATER	HIGH I	F	485	0	969	24.0
WATER	HIGH I	M	654	0	1308	39.5
STORE	LOW I	F	325	902	219	20.8
STORE	LOW I	M	497	906	209	38.1
STORE	HIGH I	F	299	928	831	13.1

APPENDIX A-2

TRIAL II - CAGE MEANS FOR THYROID GLAND CHARACTERISTICS AND SERUM THYROID HORMONE VALUES

Liquid	Cage Feed	Sex	Thyroid Weight		Thyroid 24-hr. ^{125}I Uptake		Serum Thyroid Hormone		
			(mg/rat)	(mg/100 g body wt)	(% of dose)	(% of dose/ 10 mg thyroid wt.)	T-4 (µg/100 ml)	T-3 (ng/100 ml)	T-3/T-4 (ng/100 ml/ µg/100 ml)
RSM	LOW I	F	11.7	5.7	15.22	13.00	4.1	86.5	21.3
RSM	LOW I	M	14.4	4.4	18.11	12.56	4.6	95.5	20.5
RSM	HIGH I	F	10.0	4.4	4.15	4.16	4.1	101.7	24.3
RSM	HIGH I	M	14.0	4.1	4.22	3.00	4.2	91.7	22.4
FABA	LOW I	F	7.5	3.4	13.84	18.33	4.5	99.7	22.4
FABA	LOW I	M	12.1	4.0	22.23	18.34	4.7	90.0	19.3
FABA	HIGH I	F	9.1	4.4	6.23	6.82	3.5	74.7	22.3
FABA	HIGH I	M	12.7	3.7	4.13	3.23	5.3	98.7	18.5
BRON	LOW I	F	11.0	5.1	25.32	23.30	4.1	94.0	23.1
BRON	LOW I	M	11.8	3.5	22.28	19.17	4.9	110.7	22.6
BRON	HIGH I	F	9.2	4.7	3.62	3.93	4.2	102.0	24.2
BRON	HIGH I	M	11.6	3.3	3.48	3.02	4.5	81.2	17.9

APPENDIX A-2 (Continued)

Liquid	Cage Feed	Sex	Thyroid Weight		Thyroid 24-hr. ^{125}I Uptake		Serum T-4	Thyroid T-3	Hormone T-3/T-4
			(mg/rat)	(mg/100 g body wt)	(% of dose)	(% of dose/ 10 mg thyroid wt)	(µg/100 ml)	(ng/100 ml)	(ng/100 ml/ µg/100 ml)
WATER	LOW I	F	12.0	6.7	49.87	41.48	3.2	106.5	32.9
WATER	LOW I	M	11.4	7.0	48.27	42.91	4.0	116.2	29.5
WATER	HIGH I	F	9.3	4.4	6.81	7.36	4.7	76.5	16.1
WATER	HIGH I	M	11.9	3.7	6.11	5.25	5.1	87.0	16.8
STORE	LOW I	F	10.1	4.7	7.75	7.51	4.4	89.2	20.7
STORE	LOW I	M	11.6	3.9	8.51	7.32	6.3	103.0	16.2
STORE	HIGH I	F	7.3	3.5	2.17	2.92	3.6	73.0	20.0

APPENDIX B

TRIAL III CAGE DATA

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APPENDIX B-1

TRIAL III - CAGE MEANS FOR TOTAL FEED, MILK AND IODINE CONSUMPTION, AND TOTAL WEIGHT GAINS

Liquid Cage	Feed	Feed Intake (g/cage/ week)	Milk Intake (ml/cage/ week)	Iodine Intake (µg/cage/ week)	Weight Gains (g/cage/ week)
RSM-H	LOW I	297	698	121	23.1
RSM-H	LOW I	308	670	115	21.7
RSM-H	LOW I	285	727	119	22.5
FABA-H	LOW I	282	728	210	20.8
FABA-H	LOW I	301	728	213	23.9
FABA-H	LOW I	309	703	208	23.0
RSM-M	LOW I	300	673	72	23.5
RSM-M	LOW I	295	718	72	22.9
RSM-M	LOW I	308	708	74	23.2
FABA-M	LOW I	271	672	123	20.0
FABA-M	LOW I	321	696	133	25.5
FABA-M	LOW I	305	703	132	24.9

APPENDIX B-1 (Continued)

Liquid	Cage	Sex	Feed Intake (g/cage/week)	Milk Intake (ml/cage/week)	Iodine Intake (µg/cage/week)	Weight Gains (g/cage/week)
RSM-L		LOW I	306	695	53	22.9
RSM-L		LOW I	298	715	51	24.0
RSM-L		LOW I	288	706	50	22.8
FABA-L		LOW I	288	708	55	23.9
FABA-L		LOW I	334	706	62	26.1
FABA-L		LOW I	313	725	59	24.2
WATER		LOW I	389	0	58	20.4
WATER		LOW I	388	0	58	21.5
WATER		LOW I	398	0	60	20.6
RSM-H		HIGH I	329	718	165	25.5
RSM-H		HIGH I	301	712	157	24.3
RSM-H		HIGH I	314	731	162	22.9
FABA-H		HIGH I	306	695	245	24.9
FABA-H		HIGH I	302	740	255	24.5
FABA-H		HIGH I	299	711	245	23.5

APPENDIX B-1 (Continued)

Liquid	Cage Sex	Feed Intake (g/cage/ week)	Milk Intake (ml/cage/ week)	Iodine Intake (µg/cage/ week)	Weight Gains (g/cage/ week)
RSM-M	HIGH I	279	718	106	21.2
RSM-M	HIGH I	275	737	106	23.0
RSM-M	HIGH I	287	705	108	23.8
FABA-M	HIGH I	303	677	167	21.6
FABA-M	HIGH I	321	711	177	26.0
FABA-M	HIGH I	296	725	171	23.4
RSM-L	HIGH I	291	705	88	20.7
RSM-L	HIGH I	297	694	89	25.3
RSM-L	HIGH I	310	706	93	24.0
FABA-L	HIGH I	283	712	91	21.3
FABA-L	HIGH I	309	720	99	24.7
FABA-L	HIGH I	321	703	102	24.3
WATER	HIGH I	373	0	104	20.2
WATER	HIGH I	388	0	109	21.4
WATER	HIGH I	396	0	111	21.5

APPENDIX B-2

TRIAL III - CAGE MEANS FOR THYROID GLAND CHARACTERISTICS

Cage		Thyroid Weight		Thyroid 48-hr. ^{131}I Uptake	
Liquid	Feed	Absolute (mg/ rat)	Relative (mg/100 g body wt.)	Absolute (% of dose)	Relative (% of dose/ 10 mg thyroid wt)
RSM-H	LOW I	8.1	3.9	24.98	30.72
RSM-H	LOW I	9.7	4.8	25.93	27.54
RSM-H	LOW I	13.3	5.9	33.58	27.32
FABA-H	LOW I	10.0	5.0	28.29	28.30
FABA-H	LOW I	8.2	3.8	20.31	24.45
FABA-H	LOW I	9.8	4.7	21.59	21.71
RSM-M	LOW I	10.4	4.8	51.08	50.17
RSM-M	LOW I	13.9	6.7	44.99	32.80
RSM-M	LOW I	12.3	5.8	57.71	47.36
FABA-M	LOW I	6.6	3.4	26.90	43.99
FABA-M	LOW I	8.6	3.8	27.84	32.38
FABA-M	LOW I	10.7	5.0	30.70	28.55

APPENDIX B-2 (Continued)

Cage		Thyroid Weight		Thyroid 48-hr. ^{131}I Uptake	
Liquid	Feed	Absolute (mg/rat)	Relative (mg/100 g body wt.)	Absolute (% of dose)	Relative (% of dose/ 10 mg thyroid wt)
RSM-L	LOW I	15.0	7.3	46.03	31.06
RSM-L	LOW I	12.4	5.7	51.62	43.42
RSM-L	LOW I	14.0	6.8	60.73	43.27
FABA-L	LOW I	11.6	5.4	46.44	40.08
FABA-L	LOW I	12.0	5.2	48.86	41.24
FABA-L	LOW I	13.6	6.2	62.97	48.76
WATER	LOW I	12.8	6.4	43.93	34.59
WATER	LOW I	15.5	7.5	69.65	44.72
WATER	LOW I	14.7	7.6	62.76	41.90
RSM-H	HIGH I	12.8	5.7	26.54	20.57
RSM-H	HIGH I	10.6	4.9	27.08	26.46
RSM-H	HIGH I	9.7	4.6	20.33	21.00
FABA-H	HIGH I	11.0	5.0	15.96	15.12
FABA-H	HIGH I	8.8	4.0	18.33	20.87
FABA-H	HIGH I	10.2	4.9	19.23	18.62

APPENDIX B-2 (Continued)

Cage		Thyroid Weight		Thyroid 48-hr. ^{131}I Uptake	
Liquid	Feed	Absolute (mg/rat)	Relative (mg/100 g body wt.)	Absolute (% of dose)	Relative (% of dose/ 10 mg thyroid wt)
RSM-L	LOW I	15.0	7.3	46.03	31.06
RSM-L	LOW I	12.4	5.7	51.62	43.42
RSM-L	LOW I	14.0	6.8	60.73	43.27
FABA-L	LOW I	11.6	5.4	46.44	40.08
FABA-L	LOW I	12.0	5.2	48.86	41.24
FABA-L	LOW I	13.6	6.2	62.97	48.76
WATER	LOW I	12.8	6.4	43.93	34.59
WATER	LOW I	15.5	7.5	69.65	44.72
WATER	LOW I	14.7	7.6	62.76	41.90
RSM-H	HIGH I	12.8	5.7	26.54	20.57
RSM-H	HIGH I	10.6	4.9	27.08	26.46
RSM-H	HIGH I	9.7	4.6	20.33	21.00
FABA-H	HIGH I	11.0	5.0	15.96	15.12
FABA-H	HIGH I	8.8	4.0	18.33	20.87
FABA-H	HIGH I	10.2	4.9	19.23	18.62

APPENDIX B-2 (Continued)

Liquid Cage	Feed	Thyroid Weight Absolute (mg/rat)	Thyroid Weight Relative (mg/100 g body wt.)	Thyroid 48-hr. ^{131}I Uptake Absolute (% of dose)	Thyroid 48-hr. ^{131}I Uptake Relative (% of dose/ 10 mg thyroid wt)
RSM-M	HIGH I	9.2	4.5	33.05	35.70
RSM-M	HIGH I	9.1	4.4	27.62	34.61
RSM-M	HIGH I	8.4	4.0	32.79	40.53
FABA-M	HIGH I	7.1	3.4	27.23	40.00
FABA-M	HIGH I	9.7	4.3	15.53	16.07
FABA-M	HIGH I	8.4	4.0	22.33	26.19
RSM-L	HIGH I	9.6	4.8	31.61	32.40
RSM-L	HIGH I	10.3	4.7	27.52	26.79
RSM-L	HIGH I	9.9	4.6	34.02	35.87
FABA-L	HIGH I	10.9	5.4	34.93	32.17
FABA-L	HIGH I	10.7	4.7	39.32	36.30
FABA-L	HIGH I	9.6	4.4	32.71	35.12
WATER	HIGH I	9.4	4.7	32.52	39.09
WATER	HIGH I	8.5	4.3	26.37	30.94
WATER	HIGH I	9.5	4.6	36.64	38.57

APPENDIX B-3

TRIAL III - CAGE MEANS FOR SERUM THYROID HORMONE AND THYROID ^{131}I HALF-LIFE

Cage Liquid	Feed	Serum T-4 (µg/100 ml)	Thyroid T-3 (ng/100 ml)	Hormone T-3/T-4 (ng/100 ml/ µg/100 ml)	Thyroid ^{131}I Rate of ^{131}I loss (% of dose/ hr)	Output Thyroid ^{131}I Half-life (days)
RSM-H	LOW I	3.7	77.0	20.8	0.143	2.10
RSM-H	LOW I	3.1	52.0	17.3	0.120	2.52
RSM-H	LOW I	4.7	114.5	24.1	0.130	2.31
FABA-H	LOW I	4.0	76.2	19.2	0.163	1.85
FABA-H	LOW I	2.9	[illegible]0.2	22.0	0.081	3.72
FABA-H	LOW I	4.8	113.7	23.4	0.100	2.99
RSM-M	LOW I	3.3	81.5	25.2	0.288	1.04
RSM-M	LOW I	3.4	111.2	32.9	0.150	2.01
RSM-M	LOW I	2.7	94.7	34.8	0.194	1.56
FABA-M	LOW I	4.0	82.5	20.8	0.179	1.68
FABA-M	LOW I	3.5	70.2	25.0	0.107	2.81
FABA-M	LOW I	3.4	90.7	27.3	0.137	2.20

APPENDIX B-3 (Continued)

Cage				Hormone	Thyroid ^{131}I Output	
Liquid	Feed	Serum T-4 (µg/100 ml)	Thyroid T-3 (ng/100 ml)	T-3/T-4 (ng/100 ml/ µg/100 ml)	Thyroid Rate of ^{131}I Loss (% of dose/ hr)	Thyroid ^{131}I Half-life (days)
RSM-L	LOW I	3.9	97.2	25.5	0.151	1.99
RSM-L	LOW I	3.4	110.5	33.4	0.211	1.42
RSM-L	LOW I	3.0	76.5	25.6	0.255	1.18
FABA-L	LOW I	3.0	79.2	27.6	0.203	1.48
FABA-L	LOW I	3.1	83.5	27.9	0.204	1.48
FABA-L	LOW I	2.8	84.5	29.9	0.201	1.50
WATER	LOW I	3.6	119.0	35.9	0.195	1.54
WATER	LOW I	3.8	86.2	23.1	0.277	1.09
WATER	LOW I	4.0	113.7	28.8	0.179	1.68
RSM-H	HIGH I	3.9	91.0	23.8	0.162	1.85
RSM-H	HIGH I	4.1	90.2	22.1	0.134	2.24
RSM-H	HIGH I	3.4	112.0	32.7	0.127	2.38
FABA-H	HIGH I	3.3	103.5	30.9	0.082	3.67
FABA-H	HIGH I	3.4	85.5	25.9	0.085	3.52
FABA-H	HIGH I	4.5	78.5	17.3	0.063	4.79

APPENDIX B-3 (Continued)

Cage Liquid	Feed	Serum T-4 (μg/100 ml)	Thyroid T-3 (ng/100 ml)	Hormone T-3/T-4 (ng/100 ml/ μg/ 100 ml)	^{131}I Output Thyroid Rate of ^{131}I Loss (% of dose/ hr.)	 Thyroid ^{131}I Half-life (days)
RSM-M	HIGH I	4.2	110.0	26.1	0.247	1.22
RSM-M	HIGH I	3.2	85.7	27.2	0.157	1.92
RSM-M	HIGH I	3.5	79.2	22.8	0.180	1.67
FABA-M	HIGH I	4.1	75.7	18.1	0.223	1.35
FABA-M	HIGH I	2.4	86.0	41.7	0.061	4.96
FABA-M	HIGH I	3.3	82.5	28.0	0.114	2.63
RSM-L	HIGH I	3.8	98.7	25.5	0.209	1.44
RSM-L	HIGH I	3.1	62.0	19.6	0.151	1.99
RSM-L	HIGH I	3.6	76.5	21.1	0.145	2.08
FABA-L	HIGH I	4.1	97.7	23.7	0.190	1.59
FABA-L	HIGH I	3.2	63.0	19.0	0.210	1.43
FABA-L	HIGH I	4.5	82.0	18.4	0.153	1.96
WATER	HIGH I	4.6	109.0	23.6	0.233	1.29
WATER	HIGH I	3.4	94.2	29.5	0.147	2.04
WATER	HIGH I	4.1	95.5	26.1	0.162	1.86

APPENDIX C

TRIALS II AND III COW DATA

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APPENDIX C-1

TRIAL II - COW BACKGROUND DATA

Cow No.	Feed	Birthdate	Lact. No.	Prev. BCA	Calved	Day of Lactation*	Group Average Day Of Lactation
828	RSM	20/8/68	4	160	4/3/74	169	148
941	RSM	29/11/69	3	191	19/3/74	154	
005	RSM	2/2/70	3	143	20/4/74	122	
914	FABA	29/4/69	3	177	24/3/74	149	143
205	FABA	17/1/72	1	-	1/4/74	[illegible]	
110	FABA	14/2/71	2	164	1/4/74	141	
726	BRON	26/5/67	4	132	15/3/74	158	150
149	BRON	2/11/71	1	-	23/3/74	150	
212	BRON	22/3/71	1	-	31/3/74	142	

* Day of lactation refers to day as of start of trial - Aug. 20/74.

APPENDIX C-2

TRIAL II - 5 WEEK PRODUCTION DATA (AUG. 20/74 to SEPT. 25/74)

Cow No.	Feed	Milk Prod. (kg)	Group Average Milk Prod. (kg)	% Fat	Group Average % Fat	% Prot.	Group Average % Prot.	% SNF	Group Average % SNF
828	RSM	1071.6		2.60		2.92		7.89	
941	RSM	882.4	990.2	2.75	2.39	3.16	3.12	8.32	8.26
005	RSM	1016.7		1.82		3.28		8.56	
914	FABA	987.9		3.33		2.98		8.11	
205	FABA	605.7	785.0	2.80	3.02	3.16	3.06	8.16	8.16
110	FABA	761.3		2.92		3.03		8.21	
726	BRON	730.6		3.37		3.01		7.51	
149	BRON	657.1	641.2	2.46	3.14	2.95	3.11	8.08	8.24
212	BRON	535.8		3.58		3.37		9.12	

APPENDIX C-3

TRIAL III - COW BACKGROUND DATA

Cow No.	Feed	Salt	Birthdate	Lact. No.	Prev. BCA	Calved	Day of Lactation*	Group Average Day of Lactation
941	RSM	H	29/11/69	3	191	19/3/74	233	168
123	RSM	H	12/5/71	2	130	27/5/74	164	
016	RSM	H	11/6/70	3	149	21/7/74	109	
914	FABA	H	29/4/69	3	177	24/3/74	228	179
121	FABA	H	13/4/71	2	106	29/5/74	162	
727	FABA	H	8/6/67	4	157	13/6/74	147	
110	RSM	M	14/2/71	2	164	1/4/74	220	171
945	RSM	M	6/12/69	3	158	7/5/74	184	
715	RSM	M	5/3/67	6	130	19/7/74	111	
149	FABA	M	2/11/71	1	-	23/3/74	229	187
135	FABA	M	4/9/71	1	-	2/5/74	189	
131	FABA	M	7/8/71	1	-	15/6/74	145	
908	RSM	L	20/2/69	3	162	10/4/74	211	174
005	RSM	L	2/2/70	3	143	20/4/74	201	
936	RSM	L	13/9/69	3	156	27/7/74	106	
153	FABA	L	3/12/71	1	-	17/7/74	209	186
606	FABA	L	20/1/66	6	138	22/4/74	199	
147	FABA	L	29/10/71	1	-	4/6/74	156	

*Day of lactation refers to day as of start of trial - Nov. 7/74.

APPENDIX C-4

TRIAL III - 5 WEEK PRODUCTION DATA (NOV. 7/74 - DEC. 12/74)

Cow No.	Feed	Salt	Milk Prod. (kg)	Group Average Milk Prod. (kg)	% Fat	Group Average % Fat	% Prot.	Group Average % Prot.	% SNF	Group Average % SNF
941	RSM	H	622.1	627.7	3.50	3.42	3.18	3.09	8.12	8.37
123	RSM	H	562.5		3.26		3.15		8.74	
016	RSM	H	698.6		3.50		2.95		8.26	
914	FABA	H	757.1	687.2	3.94	3.08	3.20	2.97	7.82	8.26
121	FABA	H	643.9		3.19		2.97		8.71	
727	FABA	H	660.5		2.12		2.74		7.64	
110	RSM	M	606.1	674.4	3.57	3.67	3.15	3.14	8.29	8.11
945	RSM	M	634.8		4.03		3.27		8.41	
715	RSM	M	782.2		3.40		2.99		8.50	
149	FABA	M	517.6	526.0	2.98	3.34	3.02	3.18	8.38	8.63
135	FABA	M	504.0		3.70		3.34		8.88	
131	FABA	M	556.3		-		-		-	
908	RSM	L	608.5	802.1	4.68	3.75	3.66	3.36	8.62	8.48
005	RSM	L	769.4		3.56		3.32		8.56	
936	RSM	L	1028.4		3.02		3.10		8.26	
153	FABA	L	501.3	532.0	3.90	3.32	3.25	3.26	8.38	8.39
606	FABA	L	603.1		3.38		3.25		8.22	
147	FABA	L	491.6		2.69		3.28		8.57	